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# THE EDITOR'S VIEWPOINT

On page (53) Bodecker leads a discussion of the "Illusory Sheath of Neuman." Dr. Bodecker's paper is presented in a style which has not been used in the *Journal of Dental Research*. The author's original manuscript was sent to five consultants in the field of histology and pathology. Each consultant was given the opportunity of presenting his discussion and then Dr. Bodecker wrote his summary and rebuttal for the discussion. The technic was suggested by Dr. Bodecker for use in discussions of papers of controversial nature and it appears evident that some facts were brought out in discussion that might have been overlooked without this type of prepublication evaluation. The same technic has been used in the *Journal of Dental Education*, in the section of Licensure and Examination under the editorship of Dr. Bodecker.

"Experimental Investigation of Referred Pain" (p. 3) by Hutchins and Reynolds from the Bureau of Medicine and Surgery, USN, gives the results of an experimental study, which was devised to test a hypothesis on referred pain previously presented by the authors. The results seem to justify their theory that a subliminal field may be established in an area of former injury, and that pain may be referred from a physiologically related region to the region of previous injury. The authors' result, which showed pain sensation produced at the site of recent dental work in 92.8% of individuals in whom the nasal mucosa was stimulated, may offer the clue to the many "idiopathic" pains.

On page 47 Shaw gives the results of an investigation of the role of mechanical factors in carious lesions of the cotton rat molar. Earlier studies indicated that the cotton rat molar is more like the human tooth in its susceptibility and reaction to the carious process, than is the tooth of the white rat. The findings presented by Shaw in this paper indicate that mechanical factors do not play a significant role in caries of the cotton rat as indicated by the extraction of the opposing teeth.

Coolidge, of the Zoller Memorial Clinic, has devised a method for the chemical measurement of caries activity in the enamel (p. 43). This method was devised to measure the activity of the caries in enamel in accessible areas and is based on a measurement of the phosphate in the saliva taken from the area under investigation. This adds another tool for the investigator of the process of dental caries.

Stephan and Hemmens present 3 papers giving (15) results of studies in pH changes produced by pure culture of oral microorganisms. They studied the effect of varying the microbic cell concentration because caries is usually produced in areas of very high concentration of microorganism, i.e., in areas of plaque formation. They also compared the effects of different microorganisms and different substrates and the effect of mixing certain microorganisms. Their findings indicate that the streptococci yeast, and sarcinae may prevent lactobacilli from reaching their lower pH, in vitro. They also found that a yeast was the only one of 17 microorganisms that showed pH changes proportional to the cell concentration. From their investigation it is evident that the ability of a microorganism to produce acids should not be the sole consideration in determining its possible role in caries production but also the inability to consume acid and the inability to produce alkaline substance must be given proper evaluation. These 3 papers on fundamental studies of oral microorganisms and their possible effect on dental caries production are published together because of their close inter-relationship. They may be considered as an entity to the readers profit.

Volker (p. 9) has studied the clearance of glucose from the oral cavity. His study gave evidence that the concentrations of

reducing substances, calculated as glucose, are at their maximum within 5 minutes and then return to normal after 30 minutes. Volker's results would indicate that dental caries activity is at its greatest within the first 5 minutes after ingestion of refined carbohydrate and that the activity in the oral cavity is reduced to an ineffective level after about 30 minutes. Should such findings be a warning to those who advocate brushing the teeth before breakfast and before going to bed as a preventive against dental caries and do they suggest that "three pauses that refresh" may give as much acid activity as three meals?.

Ellison and Halpert (p. 79) give the chemical changes in dentin following ageing and peripheral irritation. This paper was presented by the two authors in partial fulfillment of requirement of the D.D.S. degree at Columbia University. The analyses were conducted on 1 deciduous tooth and 18 permanent teeth and show that there are definite differences in composition between the normal dentin and sclerotic dentin. The substance which is responsible for the change was not determined by the authors.

Brown and Cruickshank of Tufts Medical College (p. 83) present further work on the effect of glycerite of hydrogen peroxide on the bacteria of the normal mouth. They compared the activity of this material with hexylresorcinol. Their findings indicate that glycerite of hydrogen peroxide is irritating than hexylresorcinol and causes a greater reduction in the number of oral organisms immediately and over a longer period of time.

Berg, Burrill and Fosdick (p. 67) are continuing their studies on the chemical effects in periodontal disease and now have devised a putrefactive index of periodontal disease. By the use of this index, they propose to evaluate the "periodontal future" of patients in a manner similar to that employed in dental caries control work with the use of lactobacillus counts and other biological yardsticks.

Applebaum (p. 73) has studied the development of rat crowns and jaws by the use of soft x-rays and has been able to demonstrate changes which were overlooked in previous studies with hard x-rays. Applebaum found no fixed point in the development of teeth and jaws of the rats. The excentric growth of the rat molars is illustrated.

A second edition of Grossman's "*Root Canal Therapy*" (Philadelphia, Lea and Febiger: 1946) has been published. The author gives the pathologic, anatomic and bacteriologic background before discussing diagnosis and treatment. The material is well presented although most of the photomicrographs are poor. The bibliographies given at the ends of the chapters appear to be well selected. One of the few points we would question is the advisability of spraying ethyl chloride on a tooth as a thermal diagnostic test. The author, himself, pointed out the limits of tolerance to temperature changes. The author appears reluctant to admit possible advantages of sulfonamides and antibiotics although he recognizes the chemotraumatic effects of many of the older drugs. The book should be valuable to dentists anticipating root canal therapy as part of their practice and reading by others might aid in eliminating prejudice based in evidence from improperly executed root canal therapy.

*Medical Research* (Philadelphia, J. B. Lippincott: 1946) is a 169 page symposium edited by Smith. W. C. Alvarez, M. G. Bohrod, E. M. Boyd, H. O. Calvery, S. Dew Clough, M. Fishbein, H. L. Gibson and T. Sollmann have contributed to a discussion of fundamentals of research, publicizing research and photography. The dental problems are so similar to those considered in *Medical Research* that dental investigators, graduate students, dental educators and others interested in dental as well as medical progress should find much meat in this small volume.

H. B. G. R.

# EXPERIMENTAL INVESTIGATION OF THE REFERRED PAIN OF AERODONTALGIA[1]

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AND

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***Research Division, Bureau of Medicine and Surgery, Navy Department***

Dental pain caused by changes in barometric pressure was shown by Reynolds, Hutchins, Werner and Philbrook (1946) to be, at least in the great majority of cases, a result of referred pain from an occluded paranasal (usually maxillary) sinus. A neural mechanism was proposed which explained not only all of the phenomena observed in their study but also the widely variant observations of previous investigators.

In summary, this hypothesis was as follows: The afferent nerve from a tooth, after once being painfully stimulated, as by a blow, deep cavitation, cavity preparation, over-filling resulting in occlusal trauma, pulp exposure, infection, or extraction, continues to react by means of a constant series of excitation waves for a considerable period of time (even years). After the original external stimulus has disappeared, however, this excitation subsides to a subliminal level. Thus no pain is experienced in the tooth until stimulation of an associated structure (innervated from the same segment) produces a facilitation of the excitation existing in the afferent nerve from the tooth sufficient to carry this excitation to the central nervous system where the subjective sensation of pain arises. This concept is schematically presented in the accompanying diagram (*fig. 1*).

Many modifications of such a mechanism are possible, as discussed in the previous paper.

Inasmuch as this hypothesis arose only from a statistical study of data collected without experimentation, it was thought desirable to test it by experimental procedures.

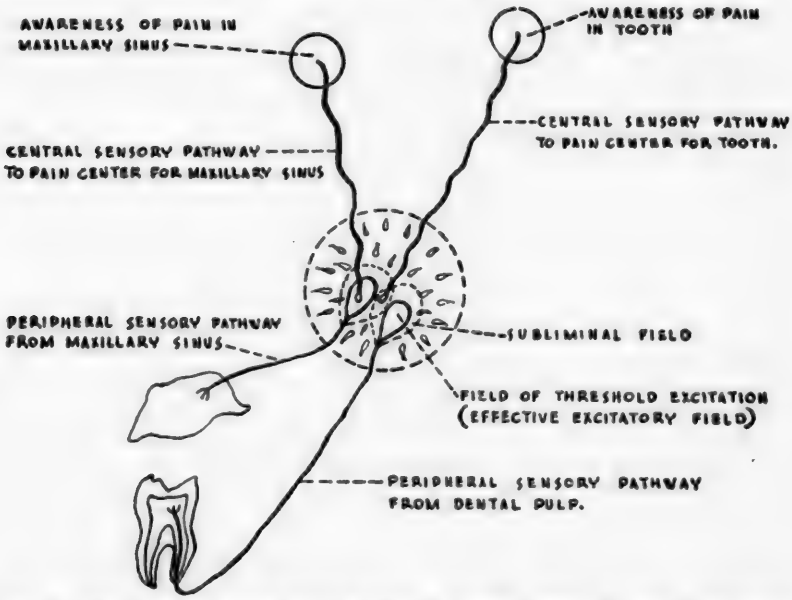
## SUBJECTS AND PROCEDURES

Fourteen subjects, 10 male and 4 female, between the ages of 14 and 52, were selected by the following criteria: (1) Dental abnormality present requiring treatment (cavity, apical abscess, etc.); (2) No previous fillings or other abnormalities present in the dentition (except as noted later); (3) Negative ENT

[1] Received for publication September 26, 1946.

[2] Present address, Medical Sciences Branch, Navy Department, Washington, 25, D. C.

examination; (4) Negative history of acute or chronic sinusitis or frequent upper respiratory infections; (5) Pathosis requiring treatment present bilaterally.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AWARENESS OF PAIN IN TOOTH
CENTRAL SENSORY PATHWAY TO PAIN CENTER FOR MAXILLARY SINUS
CENTRAL SENSORY PATHWAY TO PAIN CENTER FOR TOOTH.
PERIPHERAL SENSORY PATHWAY FROM MAXILLARY SINUS
SUBLIMINAL FIELD
FIELD OF THRESHOLD EXCITATION (EFFECTIVE EXCITATORY FIELD)
PERIPHERAL SENSORY PATHWAY FROM DENTAL PULP.

*Fig. 1.* (From U. S. Naval Medical Bulletin **46:** 845, 1946.) A tooth which contains pathosis insufficient to produce pain might be considered as giving rise to a series of subliminal or inadequate stimuli over its sensory nerve. These inadequate stimuli, on arriving at some central point of transmission, do not give rise to sufficient excitation to produce a stimulus to the central pathway and thereby subjective awareness of pain. However, if impulses are also traversing a closely associated peripheral pathway, (i.e., from the maxillary sinus) it is conceivable that these may reinforce the original impulse to such an extent that the central pathway is stimulated and subjective awareness of pain is produced. This concept may be schematically represented by a diagram such as *fig. 1* which represents the condition in which maxillary sinus pain and dental pain occur concomitantly. As is shown in the diagram the field of threshold excitation from the peripheral sensory pathway (P S P) of the maxillary sinus includes the receptor for the central sensory pathway (C S P) from the maxillary sinus. Thus, stimulation of the C S P to the pain center for the maxillary sinus occurs and awareness of pain in the maxillary sinus results. However, the field of threshold excitation resulting from stimulation of the P S P from the tooth does not include the receptor for its C S P, so no stimulation of the C S P would occur, and resulting pain would not be produced without intervention of reinforcement. This reinforcement might occur as follows: The subliminal fields (i.e., areas in which excitation is present at a level inadequate to produce stimulation) from the 2 peripheral sensory pathways overlap, including the receptor for the C S P to the tooth's pain center. This results in stimulation of the C S P and subjective awareness of pain in the tooth.

The experimental procedure consisted of:

(1) Correction of the defect. The dental defect was corrected in a manner allowing transmission of excitation by the afferent nerve (without anesthesia for fillings or under general anesthesia for extractions).

(2) Technique of cavity preparation. Cavities were prepared in teeth indicated in Table I with dental burs and excavators. In each case used, it was ascertained that subjective pain had been produced. Cavities prepared were shallow and did not approach the pulp chamber. The cavity was then sterilized with phenol, followed by alcohol, and filled with amalgam without sedative base. (The above procedure is in accord with current dental practice for shallow preparations as in this case.) None of the subjects reported painful symptoms following the dental procedure.

(3) Other dental abnormalities. In 1 individual (2 experiments) teeth were extracted under $NO_2$—$O_2$ mixture as the dental procedure.

(4) Stimulation of the maxillary sinus. Approximately 1 week after dental treatment the subjects were subjected to insertion of a nasal speculum (taking care to avoid painful stimulation) into the nostril. The lateral nasal mucosa (beneath the middle concha) in the region of the ostium of the maxillary sinus was then stimulated by a needle prick. As dental treatment was performed bilaterally, this procedure was conducted also bilaterally, producing 2 experiments per subject.

The patient's statement was then taken, along with objective observation, as to the severity and location of pain produced.

## RESULTS

Experimental data is presented in Table I. Painful symptoms were produced in the teeth treated in 92.8% of cases on stimulation of the maxillary sinus ostium. In those cases in which only 1 tooth was treated (78.5%) pain was localized to that particular tooth. Where more than 1 tooth had been treated (19.3% pain was generalized in that quadrant. In only 71.5% of cases was pain experienced in the region of the needle prick.

## DISCUSSION

Referred pain, although a commonly observed and much discussed phenomenon, has been explained by a wide variety of mechanisms. Most of these explanations involve a hypersensitivity of the posterior horn of the gray matter of the spinal cord. The nocifensors of Lewis (1937), protopathic nerves of Head (1920), various modifications of the axon reflex, and many other hypotheses are designed to explain this hypersensitivity. It is the belief of the author that the mechanism proposed here (i.e., summation of sensory stimuli from 2 afferent nerves) could be responsible for this identical effect without the necessity for introduction of hypothetical structures and little known mechanisms. Such a possibility was suggested by Erlanger in the discussion of Pollock and Davis' work (1935) on referred pain from the viscera. Heinbecker and Bishop stated that the central summation of impulses traveling the afferent pain pathway is responsible for the intensity of pain and, in fact, the sensation of pain itself. Although the summation referred to by Heinbecker and Bishop is temporal, spatial summation should be equally effective.

The hypothesis that an injured nerve may continue to give rise to waves of

TABLE I

| SUBJECT | | DENTAL TREATMENT | | TIME BETWEEN TREATMENTS[b] | PAIN PRODUCED AT SITE OF NEEDLE PRICK | PAIN LOCALIZED IN TOOTH TREATED |
|---|---|---|---|---|---|---|
| Sex | Age | Tooth | Proc.[a] | | | |
| | | | | *days* | | |
| M | 23 | 3 | II | 9 | + | + |
| | | 14 | I | 9 | + | + |
| M | 14 | 4 | I | 13 | + | + |
| | | 14 | I | 13 | + | + |
| M | 52 | 3 | I | 15 | − | + |
| | | 14 | II | 15 | − | + |
| M | 28 | 2 | I | 12 | + | + |
| | | 13 | II | 12 | + | + |
| M | 21 | 3 | II | 14 | + | + |
| | | 14 | I | 10 | + | + |
| M | 24 | 4 | II | 16 | + | + |
| | | 14 | II | 16 | ± | + |
| M | 22 | 3 | II | 11 | + | + |
| | | 14 | I | | | |
| | | 13 | II | 10 | + | +[c] |
| M | 22 | 3 | II | 15 | − | + |
| | | 13 | I | 15 | + | + |
| F | 28 | 2 | II | | | |
| | | 3 | I | 15 | − | +[c] |
| | | 15 | I | 15 | + | − |
| F | 23 | 3 | II | 14 | + | + |
| | | 14 | I | 13 | + | + |
| F | 22 | 4 | V | 14 | + | + |
| | | 13 | II | 14 | + | + |
| M | 30 | 3 | II | 13 | − | + |
| | | 13 | I | | | |
| | | 14 | I | 13 | − | +[c] |
| M | 27 | 20 | II | 11 | + | + |
| | | 30 | I | | | |
| | | 31 | I | 10 | + | +[c] |
| F | 28 | 3 | Ext. | 33 | − | + |
| | | 13 | | | | |
| | | 14 | Ext. | 33 | + | +[c] |
| | | 15 | | | | |

[a] Fillings according to Black's (1936) classification. I—Occlusal surface. II—Mesio- or disto-occlusal surfaces. V—Gingival surface. Ext.—Extraction.

[b] Time elapsed between dental treatment and stimulation of maxillary ostium.

[c] Pain, in these cases, was generalized throughout the quadrant concerned.

excitation for a considerable period of time after the initial stimulus has ceased has been discussed by many investigators. Livingston (1943) has explained this by a vicious circle concept which involves the effect of pain on the sympathetic component to the tissues adjacent to the affected nerve ending. Some experimental results and many correlaries well known in human experience are presented for support of his theory. Prominent among these is the syndrome associated with "phantom" pain of an amputated limb.

It is believed by the authors that the mechanism suggested by Reynolds, Hutchins, Werner and Philbrook (1946), and tested here experimentally, has a wider application than the dental phenomenon on which it was based.

Commonly observed phenomena associated with dental pain which, the authors believe, are directly related to the mechanism tested here are:

(1) Dental pain arising from acute maxillary sinusitis. This phenomenon is commonly observed and recognized as such by practicing dentists. Some cases which are unrecognized may result in dental work on, or extraction of, a tooth which is not pathognomic and in continuation of pain after treatment or extraction of the presumably offending tooth.

(2) Difficulty of localization of dental pain. Another commonly observed phenomenon is that of generalized aching in an entire quadrant of the dentition due to 1 diseased tooth. This might well be explained by the presence in that quadrant of 1 or more teeth containing fillings in addition to the affected tooth. These filled teeth then would exhibit referred pain of an intensity equal to that of the pathologic one, or possibly of greater intensity, as that of the offending tooth might be subliminal as in the case of maxillary sinus stimulation.

(3) Production of pain in diseased teeth by changes in barometric pressure. It has frequently been observed that altitude tooth pain occurs in a tooth containing pathosis which has not given previous trouble. This has given rise to the belief among various investigators that reduction of barometric pressure might be used as a diagnostic aid in dentistry, without consideration of the mechanism involved in production of pain. This suggestion has merit in that just such a prediction of dental trouble to come can be obtained by this means. However, since in the majority of cases pain is localized in unoffending teeth, with previously placed fillings, or subjection to some sort of trauma, the procedure would seem impractical.

A possible correction for the problems suggested in this paper might be found in procaine block of the nerve trunk involved after completion of the dental work. Successful anesthesia of visceral pain by procaine block of the peripheral nerves to the skin in the area to which pain is referred has been reported by Heinbecker, Livingston and others. This possibility is being currently investigated. In this connection, it should be mentioned that a few of the subjects used in this this study had had previous dental work under procaine block anesthesia. In no case was pain experienced in these teeth.

## SUMMARY AND CONCLUSIONS

(1) Pain sensation was produced at the site of recent dental work in 92.8% of 28 teeth (14 human subjects) by stimulation of the nasal mucosa in the region of the maxillary sinus ostium.

(2) This evidence strengthens the hypothesis that pain referred to the teeth from the maxillary sinus involves spatial summation of the excitation of 2 separate afferent nerves.

(3) Further investigations from a standpoint of procaine block of the peripheral nerve supply to the tooth during or after the dental procedure, in an attempt to prevent or remove the "hyperesthesia," are under way.

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# SOME OBSERVATIONS ON THE CLEARANCE OF GLUCOSE FROM THE ORAL CAVITY[1, 2]

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Most authorities are in agreement that the etiology of dental caries is related to the retention of the refined carbohydrates on those surfaces of the teeth that are not self-cleansing. The subsequent breakdown of these carbohydrates by oral micro-organisms results in the formation of products that initiate the carious lesion.

If this concept of caries etiology is correct, the need for information as to the concentrations of carbohydrates remaining in the mouth following the ingestion of foods and confections is obvious. Also it would seem of considerable importance to determine the velocity at which the materials are removed from the oral cavity. Since only a limited amount of information (1) is available on either of the points, the present study was undertaken. Because methods for the detection of most carbohydrates are inadequate, it was decided to analyze for reducing substances[3] (calculated as glucose). Fortunately, most schemes of oral carbohydrate degradation assign a position of major importance to this substance. Although there is some controversy as to the existence of glucose in normal resting saliva (2, 3), the question is of academic interest since we are concerned only with the fate of added ingested glucose.

## METHODS

The Folin Malmros (4) micro method for blood sugar was adapted for salivary glucose examination. All the determinations were made with a cenco photelometer. Preliminary to the main portion of the investigation the glucose level of resting saliva 2 hours post cibum was determined in 28 male dental students 19–25 years of age and the daily variations in the salivary glucose level were established in a study of 12 individuals over a period of 6 days. The 12 individuals who participated in the latter experiment were used in the more detailed aspects of the present study.

Two hours after the morning meal a sample of unstimulated saliva was collected and a 0.1 cc. aliquot was analyzed for glucose. Each participant was given a sample of the food or confection to be tested and instructed to digest it in a normal manner. Samples of saliva were collected at intervals of 5, 15, and 30

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[3] In this paper the terms glucose and reducing substance are used interchangeably.

minutes and analyzed for glucose. A comparison of the control and experimental levels indicated the salivary glucose level at stated times after the ingestion of a particular food or confection. Included among the substances tested were chewing gum, hard mints, chewy candy, cookies, and bread and jam. Except in the first named they were given in unit quantities. The exception was made when preliminary studies indicated that a minimum of 2 sections of chewing gum was necessary if results comparable to the remainder of the foods were to be expected.

A limited amount of information was also accumulated as to the reducing substance concentration of saliva after ingesting army field rations and sucking of lollypops. The ration contained 5 hard biscuits, a dehydrated sweet powder for making a drink, and 3 pieces of hard candy. The subjects were permitted to consume the biscuits and the flavored drink at a normal rate and after a wait of 10 minutes the candy was eaten. Salivary glucose levels were recorded at the

TABLE I

*Daily glucose variation*

| NAME | 1ST DAY | 2ND DAY | 3RD DAY | 4TH DAY | 5TH DAY | AVERAGE |
|---|---|---|---|---|---|---|
| Ba | 15 | 11 | 14 | 32 | 23 | 19 |
| Bo | 20 | 16 | 14 | 8 | 15 | 14 |
| McM | 20 | 23 | 19 | 32 | 10 | 21 |
| Mi | 17 | 22 | 23 | 21 | 15 | 19 |
| Pe | 17 | 15 | 16 | 40 | 11 | 20 |
| Th | 20 | 9 | 21 | 16 | 16 | 16 |
| Zu | 21 | 11 | 11 | 15 | 26 | 17 |
| Gr | 23 | 8 | 32 | 25 | 23 | 22 |
| Mac | 19 | 21 | 23 | 16 | 13 | 18 |
| Ma | 18 | 22 | 28 | 18 | 13 | 20 |
| Pa | 16 | 20 | 25 | 33 | 18 | 22 |
| Po | 19 | 12 | 26 | 18 | 14 | 18 |

All figures are mg. %

beginning of the experiment, halfway through the biscuits and drink, 10 minutes after the biscuits and drink, halfway through the candy, and 10 minutes after the candy.

## FINDINGS

The normal salivary glucose level of 28 subjects varied form approximately 13 to 30 mg. % with an average of approximately 20 mg. %. This figure was slightly higher than that recorded by Blatt, Kern and Kourtmen (5) and is in agreement with Young (3), and it is probable that the base value includes reducing substances other than glucose.

The daily variation in resting salivary glucose of 12 individuals over a period of 5 days is seen in Table I. In only 2 instances were values of less than 10 or more than 30 mg. % observed. Individual averages ranged from approximately 15 to 23 mg. %.

Table II contains information as to the average glucose concentration of the

saliva following the ingestion of chewing gum, soft mints, hard mints, chewy candy, vanilla wafer, and bread and jam. It will be noted that the salivary glucose concentration is at an appreciable level 5 minutes after eating and except for the hard mints falls precipitiously during the next 10 minutes. A normal level is usually reached at the end of one-half hour. If we consider the concentration and rate of clearance, the picture is the least favorable in the case of chewy candy and most favorable for chewing gum.

TABLE II

*Glucose concentration of human saliva following ingestion of food and confections*

| SUBSTANCE | NO. OF SUBJECTS | AVERAGE GLUCOSE CONCENTRATION IN MG % | | | |
|---|---|---|---|---|---|
| | | 0 time | 5 minutes | 15 minutes | 30 minutes |
| Chewing gum (1) (Dentyne)..... | 28 | 19 | 484 | 147 | 26 |
| Soft Mint (2) (Birch Bit)..... | 12 | 16 | 662 | 131 | 23 |
| Hard Mint (3) (Toronto Mint)..... | 12 | 21 | 377 | 259 | 24 |
| Chewy Candy (4) (Choclette)..... | 11 | 24 | 1909 | 269 | 26 |
| Cookie (5) (Vanilla wafer)..... | 12 | 16 | 889 | 117 | 18 |
| Bread and Jam (6)..... | 12 | 25 | 1475 | 149 | 22 |

(1) Weight—3.2 g. Time of eating—30 minutes
(2) Weight—2.5 g. Time of eating—4 minutes
(3) Weight—1.9 g. Time of eating—9 minutes (average)
(4) Weight—5.9 g. Time of eating—7 minutes
(5) Weight—8.2 g. Time of eating—2.5 minutes
(6) Weight—76. g. Time of eating—3 minutes

TABLE III

*The clearance of glucose* contained in Army field ration from the oral cavity*

| SUBJECT | CONTROL | HALFWAY THRU BISCUITS AND DRINK | 10 MIN. AFTER BISCUITS AND DRINK | HALFWAY THRU CANDY | 10 MIN. AFTER CANDY |
|---|---|---|---|---|---|
| A. | 47 (0)†..... | 1920 (13.5) | 300 (41) | 2480 (58) | 255 (90) |
| B. | 28 (0)..... | 700 (10.5) | 145 (33.5) | 1480 (55) | 325 (83) |
| C. | 30 (0)..... | 1100 (8) | 365 (26) | 3480 (53) | 300 (82) |

* All glucose concentrations are recorded as mg. %.

† Figures in parenthesis represent time elapsed in minutes from the beginning of the ingestion period.

The clearance of glucose from the mouth following the consumption of army field rations is presented in Table III. It will be noted that high salivary glucose concentrations are maintained for periods of approximately 1½ hours. During this time the lowest glucose value was approximately 150 and the highest 3500 mg. %.

The clearance from the oral cavity of glucose contained in a large size (38 grms.) lollypop is seen in Table IV. Quantities of glucose not less than 700 mg. % and exceeding 6000 mg. % were recorded during the time the candy was being consumed.

## DISCUSSION

The primary objective of the present study was to accumulate general information as to the amounts of carbohydrate likely to be found in a mouth following the ingestion of typical confections and foodstuffs. It should be remembered that we have analyzed only for glucose and that in all probability the test materials contained other carbohydrates, especially sucrose and starch. Undoubtedly the products of the intermediate metabolism of the latter contributed to the observed values. However, a similar statement cannot be made for the sucrose. The need for detailed information as to the clearance of this disaccharide is apparent. Because of the physical resemblance, findings parallel to those with glucose would be anticipated.

Although it is obvious that the rate of clearance of glucose from the oral cavity will be dependent upon the initial quantity ingested, the influence of several other factors must not be overlooked. In all probability certain of these might explain the wide variability found in the members of the test group. Among the more important would be the physical and chemical nature of the food, the manner in which the food was ingested, and normal oral physiology, including the rate of salivary flow, morphology of the dental arches, and the action of the muscles of mastication. It is hoped that a continuation of the present study will elucidate the relative importance of these variables.

TABLE IV

*The clearance of glucose (mg. %) contained in lollypops from the oral cavity*

| SUBJECT | 0 TIME | 15 MIN. | 30 MIN. | 1 HOUR | 2 HOURS |
|---|---|---|---|---|---|
| A* | 27 | 6160 | 3840 | 245 | 72 |
| B | 23 | 700 | 1290 | 870 | 47 |
| C | 22 | 1290 | 5160 | 2440 | 54 |
| D | 21 | 4320 | 6160 | 1060 | 64 |

* Subject consumed candy in approximately 1 hour. Others needed between one and one quarter hours.

Recently, Stephan (6) has shown that within 5 minutes after the mouth has been rinsed with a solution containing 2.5 grams of glucose the pH of the tooth surface drops below 5.0 in caries susceptible individuals. Preliminary experiments in this laboratory indicate that such a procedure would result in a glucose level of 0.5 to 1.0%. Since the concentrations encountered with foods and confections were in this approximate range similar changes in hydrogen ion concentration would be anticipated.

## SUMMARY

The concentration of reducing substance calculated as glucose has been determined before and at varying intervals after the ingestion of various foods and confections including chewing gum, hard mints, soft mints, bread and jam, cookies and chewy candy. The maximum concentration was reached within 5

minutes and the return to the normal was completed in 30 minutes. The salivary glucose levels showed considerable variation from person to person, but in general they were the greatest after chewy candy and the least after chewing gum. A limited number of observations have also been made on the clearance of glucose contained in army field rations and lollypops.

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# STUDIES OF CHANGES IN pH PRODUCED BY PURE CULTURES OF ORAL MICRO-ORGANISMS[1]

I. Effects of Varying the Microbic Cell Concentration

II. Comparison of Different Micro-organisms and Different Substrates

III. Some Effects of Mixing Certain Micro-organisms

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## I. Effects of Varying the Microbic Cell Concentration

The present state of our knowledge concerning the roles played by different oral micro-organisms in the production of carious lesions is still quite confused. Perhaps this is partly because the in vitro conditions which have generally been employed to study individual types of micro-organisms derived from the mouth have not generally resembled the in situ conditions for micro-organisms on tooth surfaces and in carious lesions. The traditional methods employed in bacteriology for study of individual types of micro-organisms utilize only a relatively small volume of micro-organisms growing in a large amount of medium. This is not the condition found on caries-susceptible areas on teeth. Here the micro-organisms grow in concentrated masses of cells (plaques) with relatively little intercellular medium.

Munts (1) while investigating acid production from glucose by dental plaque material observed that when he diluted the plaque material, the changes in amounts of lactic acid found were not in proportion to the dilution. The question arises whether or not the study of microbic cells at the low cell concentrations of traditional bacteriological methods can give an accurate picture of what these micro-organisms do at the high cell concentrations naturally occurring on teeth. The experiments described in this paper were performed to find an answer to this question.

Since, presumably, it is the acidity which micro-organisms produce which is responsible for their ability to attack enamel and dentin in caries[4] the method

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[4] The present evidence concerning the biochemical nature of this attack is neither very detailed nor conclusive. Since the many enzymatic reactions which oral micro-organisms carry out may possibly effect the integrity of tooth substance in several ways, such as the removal of phosphate by phosphorylation, the removal of calcium by formation of soluble calcium complexes with hydroxypolycarboxylic acids (such as are formed in the "tricarboxylic acid cycle" in cellular metabolism) and the removal of proteins in teeth by proteo-

employed has been to compare the pH changes produced by different cell concentrations of oral micro-organisms when suspended in a buffer resembling saliva, and containing a limited amount of glucose as a substrate.

## MATERIALS

### *Microbic cells*

Pure cultures of micro-organisms were obtained from dental plaques in the manner previously described by Hemmens, Blayney, and Harrison (5). The micro-organisms were isolated and typed according to standard bacteriological procedures. The 1 to 2 ml. volume of cells required for experiments were grown in an appropriate medium. Blood agar in Kolle flasks was used for the cultivation of streptococci, diphtheroids, and strains of neisseria; veal infusion agar in Kolle flasks was used for staphylococci, yeast, and sarcina. Cells were harvested by removing surface growth with sterile buffered saline (pH 7.0) and were washed twice and resuspended in the same menstruum. Lactobacilli were grown in Jay's broth (pH 5.0) until sufficient growth had been obtained. Cells were separated fron the medium by centrifugation and 2 washings with the sterile buffered saline and were resuspended in the same fluid. The suspensions of micro-organisms were prepared for each experiment and in the short interval between the harvesting and the pH experiments were kept in the ice-box. The cells were centrifuged down, the fluid siphoned off, and the cells resuspended in a special buffer solution.

### *Buffer solution*

The buffer which was used in these experiments was designed to resemble saliva in regard to pH, inorganic content, and buffer capacity. However, since the carbonic acid content of saliva cannot be maintained constant throughout an experiment with the use of such a buffer in an ordinary atmosphere, it was decided to omit the $CO_2$ and to increase the amount of phosphate proportionately; the calcium content was decreased below that in saliva so that precipitation of calcium phosphate would not occur.

The stock buffer solution was made in concentrated form so that a single uniform supply would suffice for all experiments, and it was made in acid and alka-

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lytic enzymes, it seems desirable to keep these possibilities in mind, even though evidence which would establish a primary role for these reactions in the caries process is lacking.

On the other hand, most of our present experimental evidence has supported the view that the primary attack on enamel in caries is produced by the action of acids. Theoretically, in order to dissolve calcium salts from teeth, the acids which are produced during the metabolic processes of oral micro-organisms must yield a great enough concentration of hydrogen-ions (low enough pH) to combine with phosphate and hydroxyl ions ($PO_4^{=}$, $HPO_4^{=}$, and $OH^-$) at a rate which will reduce the concentration of these ions below the solubility products for the calcium salts in teeth (apatites). The available evidence indicates that this would occur at about pH 5.0 and below in the mouth. Actually pH values below 5.0 have been found in plaques and in carious lesions (2), and the occurrence of pH values below 5.0 on tooth surfaces has been found to be definitely associated with caries activity (3, 4).

line fractions in order to secure keeping qualities. These 2 fractions were mixed with distilled water in the correct proportions before each experiment. Part of the concentrated acid half was made up with glucose in order to secure a uniform concentration of this carbohydrate in all experiments. The composition of these solutions was as follows:

*Solution I (alkaline half)* (kept in paraffinned bottles)

| | |
|---|---|
| $Na_2HPO_4$ | 71.0 grams |
| KOH | 79.2 grams |
| $KH_2PO_4$ | 68.1 grams |
| Tripled distilled water | to 1000.0 ml. |

*Solution II (acid half)*

| | |
|---|---|
| $KH_2PO_4$ | 45.4 grams |
| $MgSO_4 \cdot 7H_2O$ | 3.2 grams |
| $CaSO_4 \cdot 2H_2O$ | 5.7 grams |
| HCl (concentrated solution diluted 1:10) | 100.0 ml. |
| Tripled distilled water | to 1000.0 ml. |

*Solution IIG* (acid fraction with glucose)

| | |
|---|---|
| Glucose (Pfanstiel dextrose C.P.) | 54.0 grams |
| Solution II | 100.0 ml. |
| Triple distilled water | to 500.0 ml. |

In making up the buffer solution, 1 ml. of *solution I* was pipetted into a 100 ml. volumetric flask, about 90 ml. of triple distilled water added; then 1 ml. of *solution II* was pipetted in to the flask and more water added to the 100 ml. mark on the flask, and the contents mixed. This method of mixing prevents the formation of a precipitate of calcium phosphate. In making the buffer with dextrose, 5 ml. of *solution IIG* were used in place of the 1 ml. of *solution II*. In addition to this buffer, a standard phosphate buffer, pH 7.0 was employed in an experiment to illustrate the effect of buffer capacity on pH curves. Small pyrex glass test tubes (10 x 75 mm.) were used to contain the suspensions of micro-organisms during the experiments A holder for these tubes was mounted in a serological type water bath with a motor driven shaking device so that the micro-organisms could be kept suspended during the experiments. The shaking device moved the tubes through a 2 inch path once every second, and the water bath was adjusted to 37.5°C.

### *Equipment for measuring pH*

The pH determinations were made with a specially designed glass electrode setup with the following features: 1. The electrode was small so that the pH of as little as 0.1 ml. of fluid could be measured; 2. the glass and reference electrodes had long narrow stems so that they could reach the bottom of the 75 x 10 mm. test tubes which were used to hold the microbic cells, thus requiring no transfer of the cells for pH determinations during the experiments; 3. the stems of the glass and reference electrodes were coated with wax so that the suspension of microbes adhered only to the active electrode tip but not to the stems, thus minimizing the loss of material during repeated pH determination on the small

amount of cell suspension; 4. the liquid junction for the reference electrode was made through a thread of Corning .015 glass which had been sealed into a pyrex glass capillary tube which connected with the reference electrode (the Corning .015 glass forms microscopic cracks when it cools after being sealed into the pyrex glass, and these cracks permit a liquid junction to be made without appreciably contaminating the test samples with the KCl solution used in the calomel reference electrode); and 5. a washing device was built around the electrodes. With this device distilled water could be flushed vigorously over the electrode between pH determinations and thus clean them in a few seconds time. A Beckman pH meter was used to measure pH with these electrodes. This setup permitted 20 pH determinations to be made in less than 10 minutes.

## PROCEDURE

The suspension of microbic cells was placed in a 15 ml. graduated centrifuge tube, and centrifuged at 2000 R.P.M. for 15 minutes. The supernatent fluid was siphoned off and a volume of buffer equal to the volume of the microbic cells was added. The cells were resuspended in the buffer by repeatedly drawing into and blowing out of a pipette. This gave a suspension which contained 50% packed cells by volume. The suspensions for lower concentrations of cells were made by dilution with buffer solution in suitable proportions.

The following procedure was used in experiments. A series of the small test tubes was used, and 0.1 ml. of buffer solution containing M/33 dextrose was added to each tube. Then, starting with the first 2 tubes, 0.2 ml. of microbic cell suspension was pipetted into each, thus making duplicates. The tubes were shaken to mix the buffer and cells, thereby producing a 33% concentration of cells in a M/100 dextrose solution buffer. The pH of the suspension was immediately determined with the glass electrode, and this gave the initial pH reading (0 time for the pH curve). The tubes were quickly placed in the water bath at 37.5°C. and shaken continuously. The other tubes for the experiments were treated similarly, using a suspension of cells which had been diluted with buffer to give lesser cell concentrations. The pH readings for the various cell suspensions were redetermined in five minutes, then at 10 minute intervals during the first hour, and at less frequent intervals during the next 30 hours.

## RESULTS

Experiments were run on 17 different oral micro-organisms including strains of staphylococci, streptococci, lactobacilli, yeasts, diphtheroids, neisseriae, and sarcinae. The initial substrate concentration was uniform in each experiment, but the concentration of microbic cells was varied from 1% to 33% of the total volume.

In general, it was found that only at the high cell concentrations do micro-organisms produce pH curves which resemble the pH curves which occur in plaques on teeth after carbohydrate has been added. At the relatively low cell concentrations pH curves did not resemble the pH curves in the plaques.

The results of an experiment with a strain of *Staphylococcus albus* are shown in *figs. 1* and *2*. In *fig. 1* the pH has been plotted against time so that the curves

compare the pH changes as they actually occurred. It can be seen that the concentration of cells greatly affected the resulting pH changes: with high cell con-

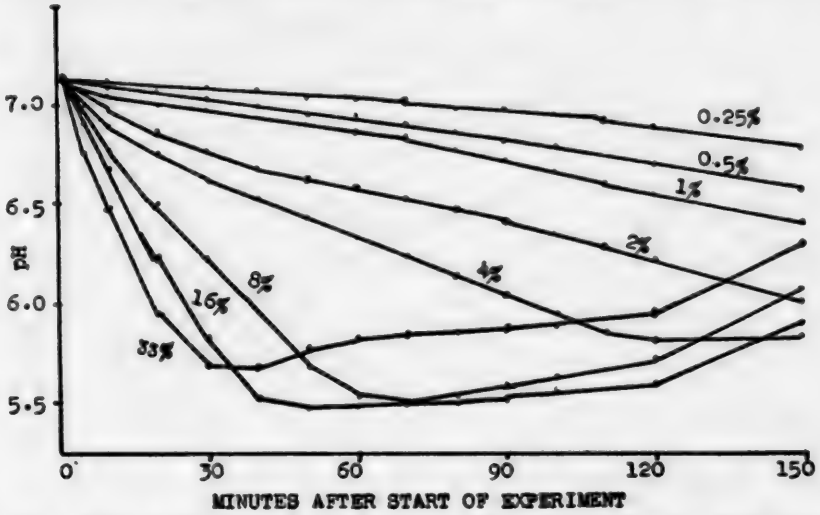


*Fig. 1.* Effect produced by varying the concentrations of staphylococcus albus cells on pH curves (substrate, M/100 glucose).

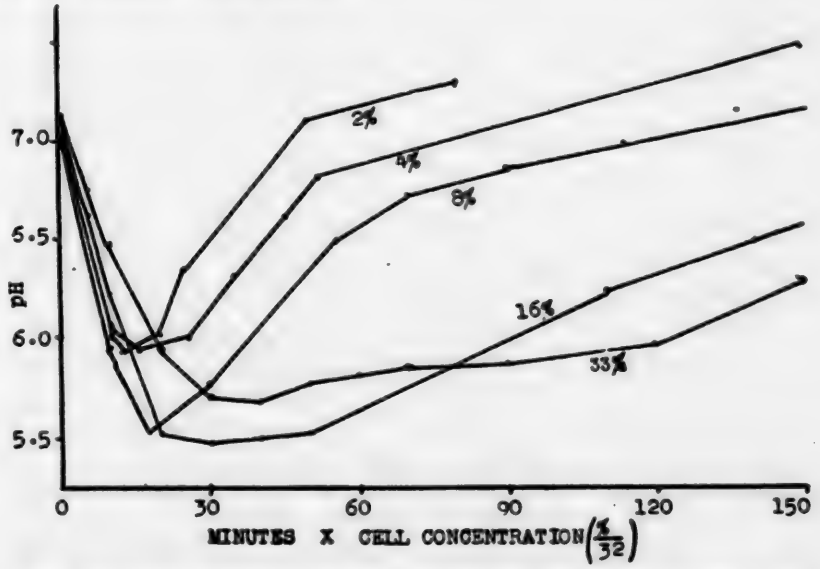


*Fig. 2.* Same data as Figure I but calculated for equal numbers of cell hours work.

centrations both the pH drop (acid production phase) and the subsequent pH rise (acid consumption phase) occur more quickly than with lower cell concentrations. But, if the time for the low cell concentrations were increased propor-

*Fig. 3.* Effect produced by varying the concentrations of neisseria cells on pH curves (substrate, M/100 glucose).

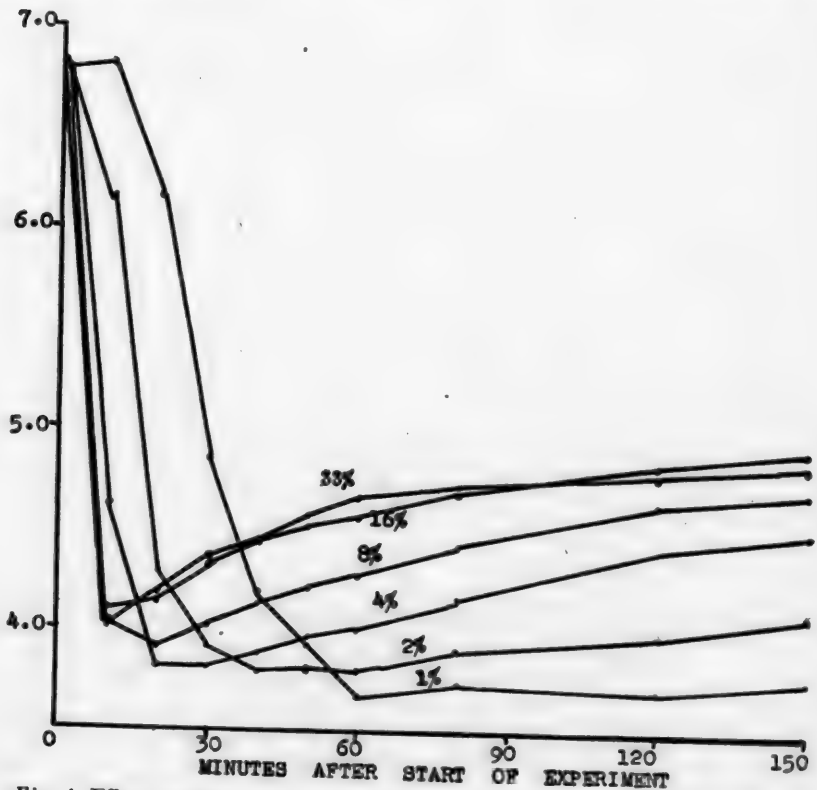


*Fig. 4.* Effect produced by varying the concentrations of lactobacillus cells on pH curves (substrate, M/100 glucose).

tionately to the decrease in cell concentration would the pH curve be the same? That this is not the case is shown in *fig. 2*. Here pH has been plotted against time multiplied by cell concentration so that the curves show pH changes produced by equivalent numbers of cell-hours work, regardless of the cell concentration. It can be seen with this method for plotting the results, that the pH drop (acid production phase) and pH rise (acid consumption phase) were not proportional to the differences in cell concentrations but were relatively quicker with the low cell concentrations than with the higher cell concentrations. It

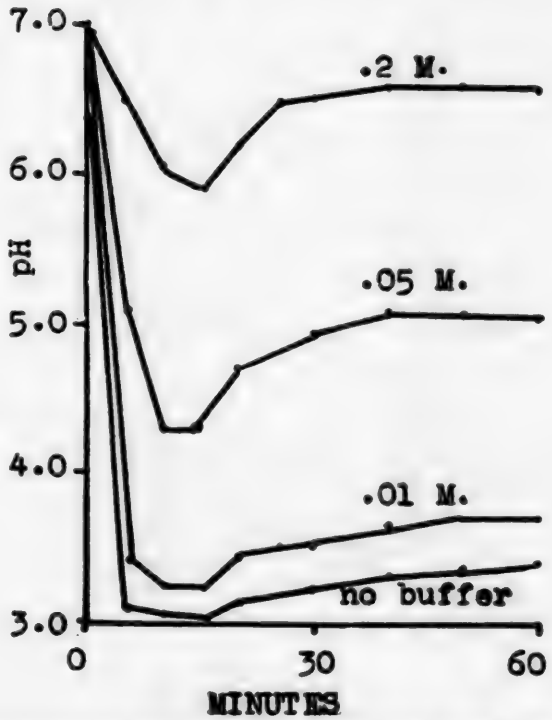


*Fig. 5.* Effect of varying the concentration of phosphate buffer on pH curves produced by yeast (substrate, M/7 glucose) (33% yeast cells by volume).

should also be noted that the lowest pH level was reached with an intermediate cell concentration, namely 16% cells.

An example of the effect of cell concentration of a micro-organism which consumes acid relatively rapidly (a strain of neisseria) is shown in *fig. 3*. This micro-organism produced acid rapidly at the 33% cell concentration but as soon as the substrate had been utilized it consumed the acid that had been produced. Both acid production and acid consumption were slow at the 1% and 2% cell concentrations so that relatively slight pH changes took place at these concentrations. It should be noted that the lowest pH level (4.4) was reached at the 8% cell concentration. Thus, paradoxically, neither the cell concentration with

the greatest rate of acid production, nor that with the least rate of acid consumption produced the lowest pH level, but rather a cell concentration in which these factors maintained a certain balance.

An example of a micro-organism which produces acid relatively rapidly and consumes it relatively slowly was found in a lactobacillus. This bacterium produced a rapid drop in pH even at the 4% cell concentration, as is shown in *fig. 4*. Here again the different cell concentrations failed to reach the same low pH level, but unlike the staphylococcus and neisseria, the lowest pH level was

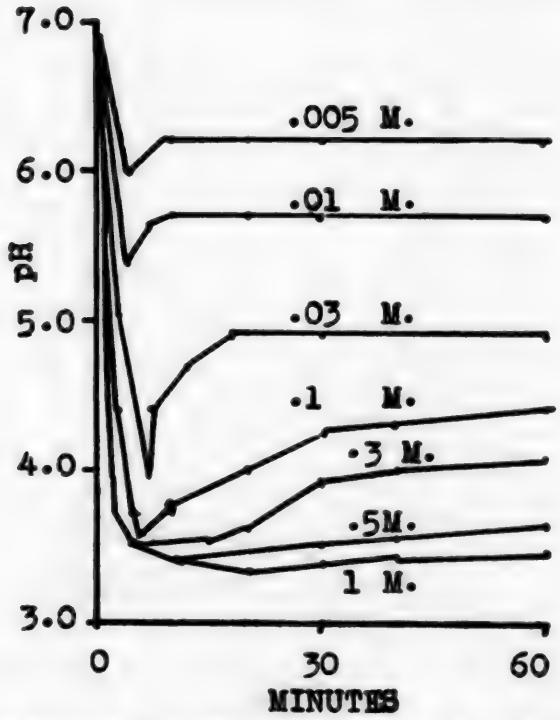


*Fig. 6.* Effect of varying the concentration of glucose on pH curves produced by yeast (33% yeast cells by volume).

produced at the lowest cell concentration (%). This difference in the lowest pH levels may be due to the relatively slow rate of acid consumption by this lactobacillus, because only at the high cell concentrations was the rate of acid consumption sufficiently rapid to produce an appreciable effect in raising the initial pH level.

Only 1 of the 17 strains of oral micro-organisms on which cell concentration experiments were done produced practically identical pH curves for all cell concentrations when pH was plotted against time multiplied by cell concentration. This was a diphtheroid. Significantly, there was practically no rise in pH (acid

consumption phase) when this organism was used, whereas in all other cases some rise in pH occurred at the higher cell concentrations.

The demonstration of the importance of cell concentration in pH studies of micro-organisms might be misleading if the effects of buffer concentration and substrate concentration were totally ignored. For this reason, separate experiments were run on a micro-organism (a yeast) in which buffer and substrate concentrations were also varied. *Figs. 5, 6,* and *7* show the effects of varying respectively the buffer concentrations, the substrate concentrations, and the cell concentrations, on the resulting pH curves. It can be seen that the lowest pH values were reached with the lowest buffer, highest substrate, and highest cell concentrations, respectively. These experiments show clearly the importance of each of these variables on the pH curves produced by micro-organisms.

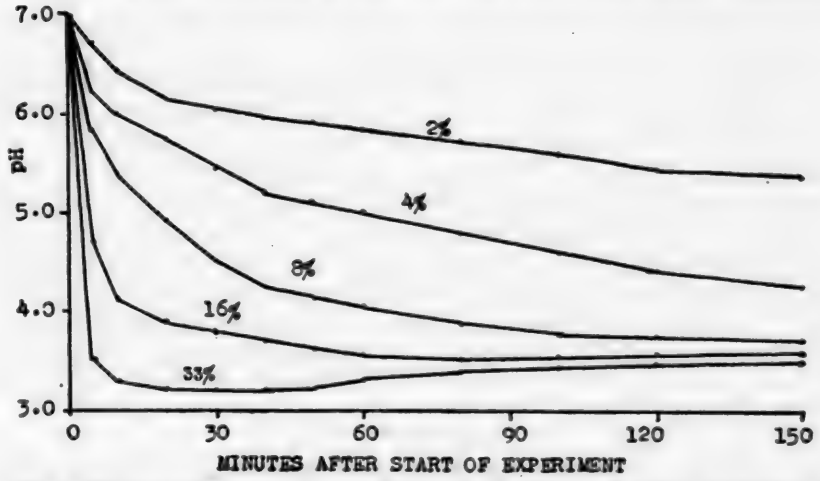


*Fig. 7.* Effect of varying the concentration of yeast cells on pH curves (substrate, glucose M/2).

## DISCUSSION

Many investigators of the relation of oral micro-organisms to dental caries have interpreted differences found in the acidogenic properties of various micro-organisms to indicate differences in the ability of the micro-organisms to produce caries. The validity of such interpretations seems to depend on the extent to which the experimental conditions employed have represented the essential conditions which occur in caries. In the usual tests for acid production from carbohydrates in culture media and saliva, a relatively small number of microbic cells act on a relatively large amount of carbohydrate in a relatively large amount of buffer. With such methods pH changes are slow, but acid gradually accumulates because the rate at which most bacteria produce acid is much greater than the rate at which they consume it, and because there is generally an excess of carbohydrate for the micro-organisms to metabolize. The pH after 24 to 72 hours of incubation may drop quite low, and under these conditions it is not sur-

prising that tooth substances placed in such media will be decalcified by bacteria isolated from mouths which have never developed caries. The actual conditions on tooth surfaces are quite different. Here the dense populations of microbic cells (plaques) can produce their maximum acidity in a matter of minutes (6) and even the relatively slow enzymatic processes may proceed at a rate which is apparent in a short period of time. Under these conditions, carbohydrates may be quickly metabolized, and unless more carbohydrate is added, the pH may return to its original level in about 1 hour (3, 4).

The experiments described here demonstrate that several different types of mouth micro-organisms are capable of producing in vitro pH changes from glucose as rapidly as are produced in natural plaques on teeth but only when the experimental microbic cell concentrations are comparable to the microbic cell concentrations on teeth.

Micro-organisms may not only produce many different acids by various interrelated enzymatic processes, but may also consume the acids. The rates of these processes may vary in different mathematical relationships when the concentration of cells is changed. Thus the pH produced by a micro-organism at low cell concentrations does not indicate what the pH will be under similar conditions at high cell concentrations. Furthermore, micro-organisms differ in the effect that varying their cell concentration produces on the pH levels which they attain. Some micro-organisms produce the lowest pH levels at high cell concentrations, some at intermediate cell concentrations and some at low cell concentrations. The differential rates of acid production with acid consumption may furnish a possible explanation of these differences. It seems clear, therefore, that one cannot ignore the quantitative factor in studying the relation of micro-organisms to caries.

## SUMMARY

The pH curves produced in a standard buffer solution containing glucose have been determined for several oral micro-organisms at cell concentrations of 1 to 33% by volume. It was found that only at high cell concentrations are the pH changes as rapid as those which occur in the microbic plaques on teeth. Furthermore, in only 1 of the 17 micro-organisms were the pH changes produced at different cell concentrations proportional to the cell concentration.

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## II. Comparison of Different Micro-organisms and Different Substrates

In the first paper of this series (1) it was found that pure cultures of several oral micro-organisms will produce pH changes with glucose which are comparable to the pH changes which occur in the microbic plaques on teeth, but only when the cell concentration of the micro-organisms in vitro approaches the high concentration of microbic cells in plaques. It seemed worthwhile, therefore, to compare the pH curves which high cell concentrations of different types of oral micro-organisms produce with some of the different substrates which may contact the teeth, in order to evaluate the roles that these different micro-organisms and substrates may play in the caries process. The following considerations governed the choice of substrates: First, since the excessive eating of highly refined carbohydrate foods has frequently been held responsible for activating the caries process, it seemed desirable to investigate the pH curves by employing several different types of carbohydrate as substrates. Second, since lactic acid which is an end produce of the glycolytic enzyme system, and which has been thought to be chiefly responsible for the acidity in caries, may itself become a substrate for further enzymatic reactions, this substance has been employed as a substrate. Third, since urea is present in the saliva and lymph at almost its level in the blood, and since urea exerts a pH effect on plaques which is the opposite of that produced by glucose, it seemed worthwhile to determine pH curves with this substance as a substrate. (There is evidence that glucose itself is not secreted in the saliva in appreciable amounts, even though it is present in the blood).

### MATERIALS AND METHOD

The buffer solutions, water bath, and equipment for determining pH employed in these experiments were the same as those described in the previous paper (1). The micro-organisms obtained from dental plaques or salivas of patients seen in the Walter G. Zoller Memorial Dental Clinic were isolated and grown as previously described (2). Forty strains of micro-organisms including actinomyces, alpha and gamma streptococci, diphtheroids, micrococci, monilia, neisseria, lactobacilli, sarcinae, staphylococci, and yeasts were used at a cell concentration of 33% by volume in these experiments. The substrates used were glucose, fructose, galactose, maltose, sucrose, and lactose (Pfansteil C. P. grades) and Merck's soluble starch, urea, and lactic acid.

### RESULTS

Since glucose is one of the most commonly used substrates in bacterial studies, experiments with this carbohydrate will be described first.

The pH curves produced by some representative strains of the different micro-organisms, with a limited amount of glucose (.01 Molar concentration) as a substrate are compared in *fig. 1*. It can be seen that there are marked differences in the pH curves produced by the various micro-organisms. For example, 1 strain of alpha streptococci (I) did not produce any observable change in pH

whereas another strain of alpha streptococci (II) produced a very rapid drop in pH during the first 5 minutes followed by a gradual rise in pH, (several other strains of alpha and gamma streptococci not shown in *fig. 1* produced pH changes between these 2 extremes). One strain of lactobacilli (I) produced an equally rapid drop in pH and reached the lowest pH of all microbes tested, but another strain of lactobacilli (II) produced a slower pH drop and did not reach as low a

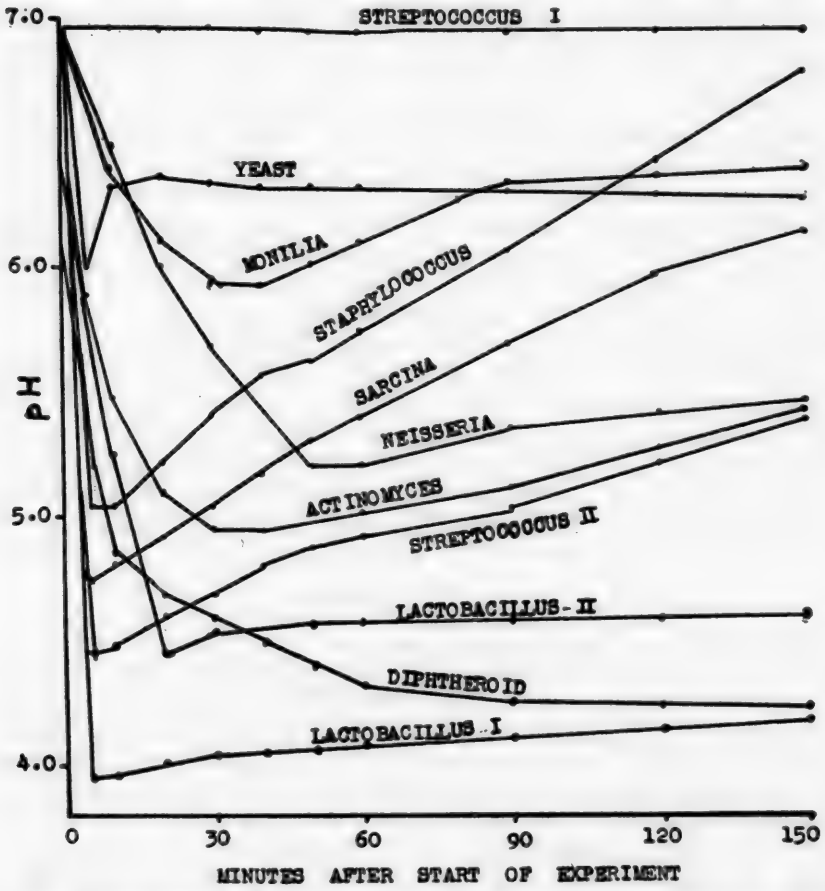


*Fig. 1.* Comparison of pH curves produced by different micro-organisms from a limited amount of carbohydrate substrate (.01 molar glucose).

pH level. A yeast produced a rapid pH drop during the first few minutes, then a rapid rise in pH during the next few minutes and after that remained at a comparatively high pH level. A monilia yeast-like micro-organism on the other hand produced a much slower drop in pH. It is interesting to note that, with the exception of a diphtheroid, every micro-organism showed some rise in pH following the initial pH drop. This evidence of the ability of the micro-organisms to

consume the acids they produce was most marked in the case of staphylococci, sarcinae and yeasts, and least marked in the diphtheroids and lactobacilli, al-

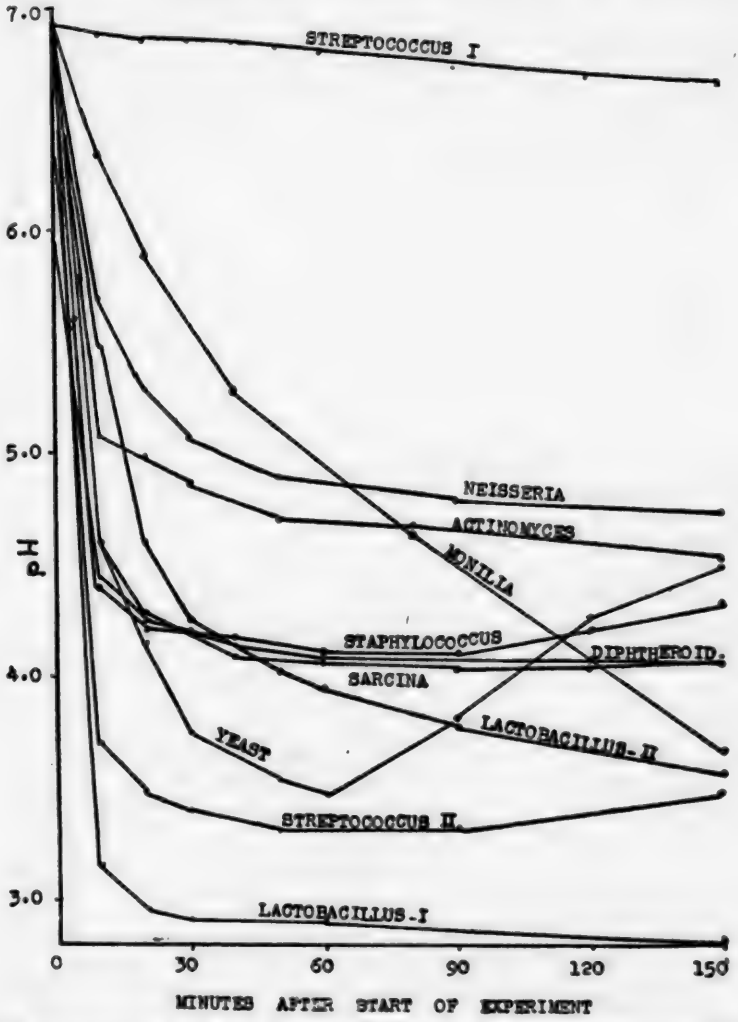


*Fig. 2.* Comparison of pH curves produced by different micro-organisms from an excess amount of carbohydrate substrate (1.0 molar glucose).

though as in the case of acid production, there were variations among different strains of the same micro-organisms.

*Fig. 2* shows the pH curves produced by the same micro-organisms when an excess of glucose (Molar concentration) was used as a substrate. The micro-organisms generally produced much lower pH levels with this increased amount of

glucose, although the difference was small for the diphtheroid, and 1 strain of streptococci again apparently failed to produce acid. Unlike the experiments with a limited amount of glucose, those with an excess amount of glucose gave curves in which the lowest pH levels were maintained throughout the experiment. The effects of acid consumption were not generally evident. Probably the dextrose was not all metabolized so that as the acids first formed were consumed, more acids could be formed to replace them. An exception to this was the marked rise in pH after the initial pH drop produced by the yeast. This indicates the ability of this micro-organism to metabolize the intermediary acidic substances of its metabolism at a relatively high rate.

TABLE I

*Comparison of increases in hydrogen-ion concentrations produced by different micro-organisms from different substrates*

(Expressed as units of drop in pH during first ten minutes of experiment)

| MICRO-ORGANISM | SUBSTRATE | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | None | Glucose | Fructose | Galactose | Sucrose | Maltose | Lactose | Starch |
| Actinomyces | 0.0 | 1.6 | 1.2 | 1.5 | 1.4 | 1.4 | 0.2 | 0.3 |
| Streptococcus (alpha) I | 0.0 | 3.1 | 3.1 | 3.1 | 0.1 | 0.3 | 0.0 | 0.3 |
| Streptococcus (alpha) II | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| Streptococcus (alpha) III | 0.0 | 2.3 | 2.3 | 2.4 | 0.4 | 0.5 | 2.1 | 0.4 |
| Streptococcus (gamma) | 0.0 | 0.5 | 0.5 | 0.6 | 0.7 | 0.7 | 0.7 | 0.6 |
| Diphtheroid | 0.4 | 1.0 | 1.0 | 1.0 | 1.1 | 0.8 | 0.4 | 0.4 |
| Lactobacillus I | 0.3 | 3.0 | 2.9 | 2.9 | 0.7 | 0.7 | 0.3 | 0.3 |
| Lactobacillus II | 0.4 | 1.0 | 2.8 | 1.2 | 1.2 | 0.7 | 1.0 | 0.4 |
| Monilia | 0.7 | 0.7 | 1.3 | 0.8 | 0.5 | 0.7 | 0.5 | 0.6 |
| Neisseria I | −0.1 | 0.6 | 0.3 | 0.3 | 0.5 | 1.0 | 0.0 | 0.0 |
| Neisseria II | −0.1 | 1.0 | 0.8 | 1.0 | 0.8 | 1.2 | 0.1 | 1.0 |
| Sarcina I | 0.0 | 2.0 | 2.0 | 1.9 | 1.3 | 1.8 | 0.1 | 0.6 |
| Sarcina II | 0.0 | 2.2 | 2.2 | 2.0 | 0.2 | 0.3 | 0.1 | 0.2 |
| Staphylococcus I | −0.1 | 2.6 | 0.3 | | | | 0.0 | 0.0 |
| Staphylococcus II | −0.1 | 2.3 | 2.2 | 2.2 | 1.9 | 0.7 | 0.0 | 0.0 |
| Yeast I | 0.1 | 2.1 | 2.6 | 0.7 | 0.1 | 2.0 | 0.1 | 0.3 |
| Yeast II | 0.0 | 2.1 | 2.5 | 1.0 | 0.2 | 1.2 | 0.2 | 0.3 |

In addition to glucose, experiments were performed with other carbohydrates as substrates, including fructose, galactose, sucrose, maltose, lactose, and soluble starch (and without carbohydrate as a control). In these experiments the carbohydrates were present in excess amounts (equivalent to molar concentration of glucose). Controls were established by omitting all types of substrate. Table I gives a comparison of the units of pH drop produced during the first 10 minutes of the experiments. This short interval of time was selected for purposes of comparison because some micro-organisms produced their maximum pH change within this interval, and it would be impractical to show complete pH curves for all experiments. It can be seen that the rapidity of the pH drops varied with different substrates as well as with different micro-organisms. In general, the

monosaccharides produced the most rapid pH changes, sucrose and maltose next, and lactose and soluble starch showed the slowest pH changes.

It is of interest to note that some micro-organisms produced pH changes in the controls (without carbohydrate). Thus on the one hand the staphylococci and neisseria produced a rise in pH while on the other hand the monilia, diphtheroids, and lactobacilli produced a fall in pH. Apparently the products of the endogenous metabolism of these micro-organisms lead to these pH changes,

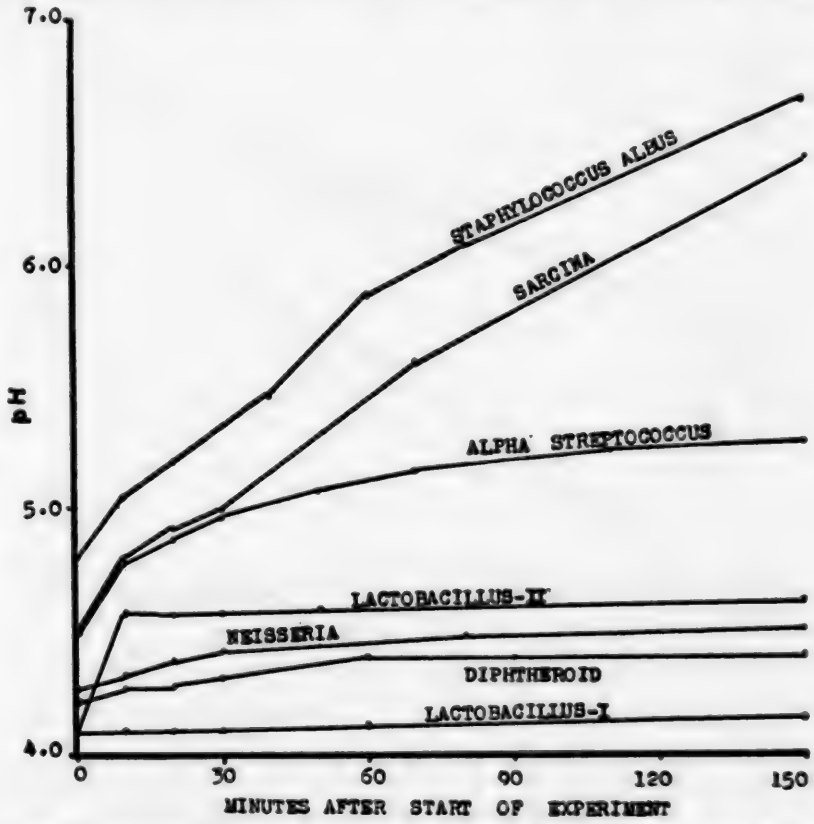


*Fig. 3.* Comparison of pH curves produced by different micro-organisms from .02 molar lactic acid.

the mechanism of which is not apparent, though possibly of considerable importance in the caries process.

It frequently has been assumed that the factor which determines the ability of micro-organisms to produce caries is their rate of acid production. The importance of the converse factor, namely the rate of acid consumption, has generally been neglected. Therefore, some pH experiments were performed with the micro-organisms using lactic acid as a substrate. *Fig. 3* gives a comparison of

pH curves produced by several types of micro-organisms with 0.02 molar lactic acid as a substrate. One can see that the various micro-organisms differed greatly in their ability to consume the acid. It is interesting to note that lactobacillus, strain I, produced practically no pH change with lactic acid as a substrate whereas lactobacillus, strain II, produced a rapid pH rise during the first 10 minutes and then did not change further. Apparently lactic acid is practically a single end product with lactobacillus I, whereas for lactobacillus II some

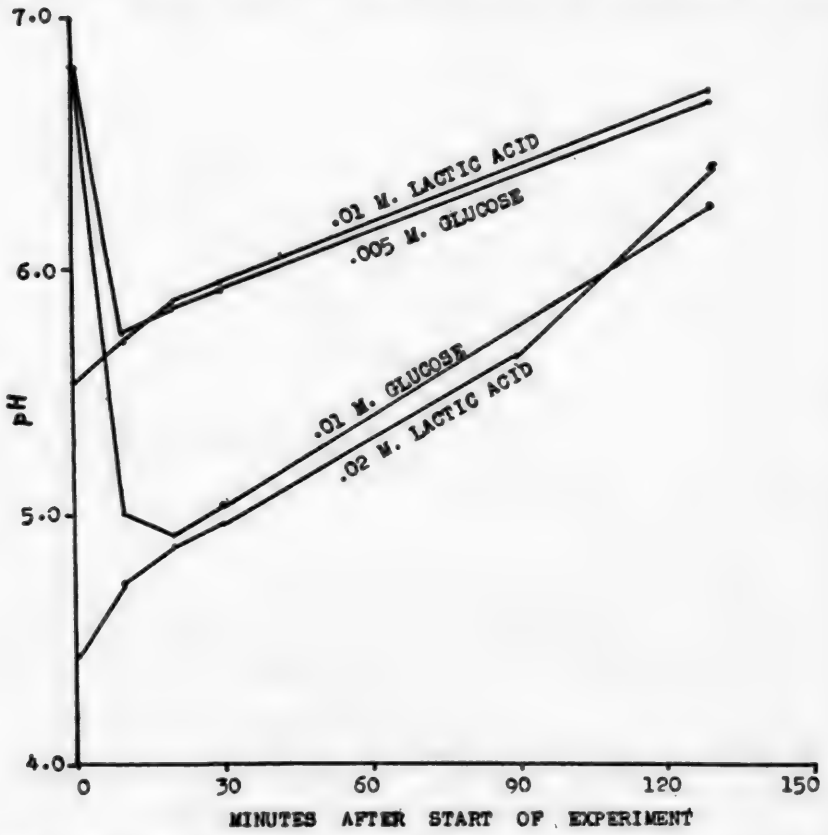


*Fig. 4.* Comparison of pH curves produced by sarcina from equivalent amounts of lactic acid and glucose.

equilibrium mixture containing some weaker acids (such as acetic) constitute the end products (see references (3, 4)).

In order to ascertain whether lactic acid is consumed at the same rate as the acids which the various micro-organisms produce from glucose, pH experiments were performed on different micro-organisms using 0.02 and 0.01 molar lactic acid for comparison with 0.01 and 0.005 molar glucose (in glycolysis 1 mole of glucose forms 2 moles of lactic acid). The result for 1 micro-organism, a sarcina,

is shown in *fig. 4*. It can be seen that with the exception of the first 10 to 20 minutes when the pH rose with lactic acid and dropped with glucose, the curves are almost identical.

In general it was found that those micro-organisms which produce a relatively rapid drop in pH with a limited amount of glucose (sarcina, lactobacilli, staphylococci and some streptococci) gave practically identical pH curves with lactic acid as with an equivalent amount of glucose after the first 10 to 20 minutes. On the other hand, those micro-organisms which produced a comparatively slow pH drop with glucose (Neisseria, monilia) and 1 strain of streptococci (II) gave

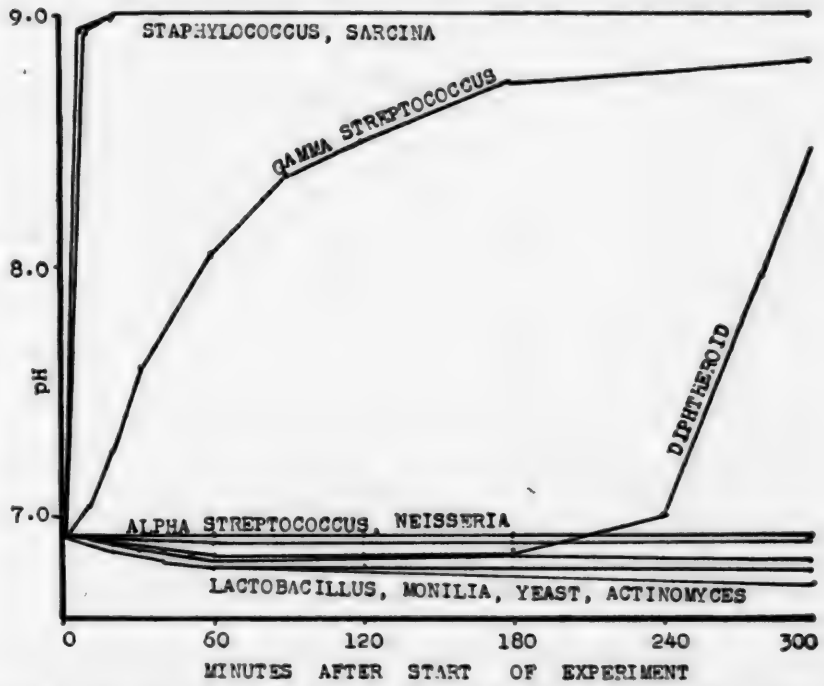


*Fig. 5.* Comparison of pH curves produced by different micro-organisms from urea (.1 molar).

pH curves with lactic acid which either ran parallel to each other or gradually converged toward the same pH level as those with glucose.

The experiments with urea as a substrate are summarized in *fig. 5*. It can be seen that an exceedingly rapid rise in pH was produced by staphylococci and sarcinae thus indicating that these micro-organisms possess the enzyme urease. An immediate though slower pH rise was produced by a gamma streptococci. No pH rise was produced by lactobacilli, alpha streptococci, yeasts, monilia, neisseria, actinomyces, thus indicating that these micro-organisms do not contain urease.

However, the diphtheroids were most interesting in that they produced no pH rise during the first 2 hours, but thereafter produced a moderately rapid rise in pH to the limiting value of 8.5. This may indicate that these micro-organisms possess only a negligible urease activity in the absence of urea, but in its presence, they may form urease in appreciable amounts. For this micro-organism, therefore urease may be an adaptive enzyme, whereas in the other micro-organisms tested it is either naturally present or absent. (Tests were also run on a few intestinal bacteria not isolated from the mouth. Of these aerobacter aerogenes and *B. proteus* showed strong urease activity whereas *B. coli* produced no pH change with urea.)

## DISCUSSION

In a previous study of intra-oral hydrogen-ion concentrations associated with dental caries activity (5), it was found that although acid was produced rapidly from glucose in the dental plaques of caries-inactive as well as caries active individuals, nevertheless, there were clear-cut differences between the pH levels reached by the plaques. Low pH levels were definitely associated with caries activity. Presumably these differences were due at least in part to differences in the micro-organisms which made up the plaques.

Since the experiments described in this paper have utilized a microbic cell concentration which approaches the microbic cell concentration in plaques, we may attempt a comparison between the pH curves produced by the different micro-organisms in vitro with the pH curves produced by plaques in situ. (Of course a strict comparison of in vitro with in situ conditions cannot be made, particularly since the effect of salivary flow in situ is to gradually neutralize and wash away acids, and in general to remove food substrates from the mouth.) First, it is apparent that a rapid drop in pH is produced from glucose by most of the micro-organisms in vitro just as it is produced by most plaques in situ. Therefore, mere rapidity in acid formation down to around pH 5.0 does not seem to be a distinguishing characteristic either among oral micro-organisms in pure culture or in plaques.

However, when a comparison is made of the ability of micro-organisms to produce pH levels below 5.0 with a limited amount of glucose as substrate, then a distinction between the micro-organisms can be made. The lactobacilli, diphtheroids, some sarcina and some streptococci were the only micro-organisms to produce such pH changes. Furthermore, if the ability to maintain as well as produce such pH levels is also considered then the sarcina and streptococci are eliminated because they consume the acid and do not maintain the low pH for the duration of the experiment. Even then the 2 strains of lactobacilli differed in their ability to maintain low pH levels produced with lactic acid. Thus these experiments indicate that the low pH levels associated with caries activity may result not only from production of acids but also from an inability of some micro-organisms to further metabolize the acids.

With an excess of glucose as substrate conditions are quite different. Here most oral micro-organisms tested can produce and maintain pH levels such as are

associated with caries activity. Under these conditions, acid consumption becomes relatively unimportant since as rapidly as acid is consumed more acid can be produced to replace it.

The fact must also be considered that some micro-organisms can produce a rise in pH levels from neutral substrates other than carbohydrates, while other micro-organisms do not produce such a change. As an example, the sarcina, staphylococci, diphtheroids, and some streptococci may counteract acidity by the production of ammonium carbonate from urea, whereas the lactobacilli, neisseria, monilia, yeasts, actinomyces, and some of the streptococci do not. Finally, the fact that some of the micro-organisms used in these experiments (diphtheroids, lactobacilli, monilia, neisseria, and staphylococci) can produce pH changes in the absence of added substrate (with products of endogenous metabolism) suggests that every enzyme system possessed by oral micro-organisms should be considered of potential importance in the caries problem.

SUMMARY

A comparison has been made of the pH curves produced by pure cultures of different types of oral micro-organisms at high cell-concentrations (33% by volume) with various carbohydrates, lactic acid and urea as substrates. The differences in the pH curves produced by different micro-organisms suggest that in addition to the ability of micro-organisms to produce acid, the inability to consume acid and the inability to produce alkaline substances are also of importance in regard to the etiology of dental caries.

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## III. Some Effects of Mixing Certain Micro-organisms

Although dental caries was one of the first diseases which was visualized as microbic in origin, nevertheless attempts to find and establish specific micro-organisms for the cause of caries have met with only a limited degree of success. W. D. Miller as early as 1891 showed that several different micro-organisms from the mouth could attack tooth substance and simulate caries in vitro, but such evidence does not constitute proof that these micro-organisms can initiate caries in vivo. The work of Kligler, Rodriguez, Bunting, Jay, Harrison, Hemmens, Arnold, Kesel, Becks and others has indicated that lactobacilli are usually associated with active caries. However, the evidence offered is insufficient to prove that these bacteria are the cause of caries. The fact that many different types of micro-organisms are always present together on tooth surfaces seems to preclude the possibility that a single kind of micro-organism can attack teeth without being influenced in one way or another by the action of other types of micro-organisms.

A criterion which has frequently been employed to indicate the ability of micro-organisms to produce caries has been to compare the amounts of acid that different oral micro-organisms produce when incubated with carbohydrates in culture media or saliva. On this basis Hansen, Fosdick and Epple (1) found that lactobacilli alone did not produce acid nearly as rapidly as a mixture of lactobacilli and yeasts. They postulated that only a mixture of micro-organisms was capable of producing acid raidly enough to cause caries. Bibby (2) has stated that streptococci are probably responsible for the production of most of the acid in caries, and that lactobacilli play only a minor role because they are so outnumbered by the streptococci in carious lesions. In their studies, acid production depended to a considerable extent on growth of the micro-organisms in the media employed. There is some question whether the culture media permitted a fair growth of all the micro-organisms to occur, that is, it is doubtful whether the quantitative factor for the micro-organisms was adequately controlled.

It therefore was desirable to determine the effect of mixing different types of oral micro-organisms with oral lactobacilli in definite proportions and at high cell concentrations in order to see if the presence of various other micro-organisms may augment or decrease the ability of lactobacilli to produce the low pH values associated with active caries.

### Materials and Method

The materials and method employed in these experiments were the same as those previously described (3, 4), except that here mixtures of pure cultures of 2 kinds of micro-organisms have been used in addition to pure cultures of single types of micro-organism. In all cases the total microbic cell concentration was 33% by volume of the cell suspension, the remainder being buffer solution and substrate.

## RESULTS

Since various yeasts and yeast-like micro-organisms are frequently found to grow on a pH 5.0 culture media commonly used for quantitative counts of lactobacilli in saliva, the effect of mixing these kinds of micro-organisms was investigated. *Fig. 1* shows 3 pH curves produce in buffer with 0.03 Molar glucose as substrate by a yeast, a lactobacillus, and a mixture of equal volumes of yeast and lactobacillus. It can be seen that the mixture has given a pH curve intermediate between the curves of the 2 micro-organisms alone. It is clear then, that with

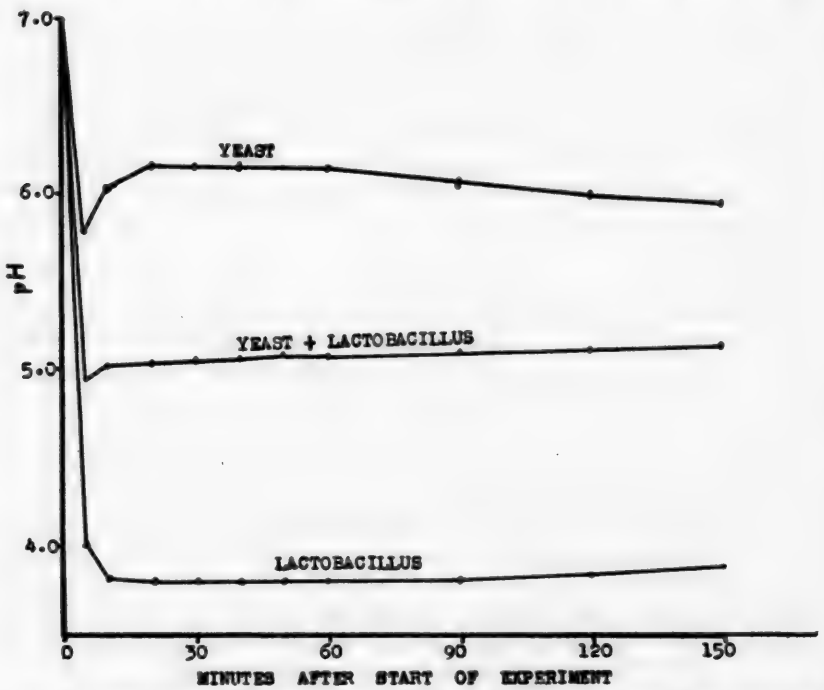


*Fig. 1.* Comparison of pH curves produced by a mixture of yeast and lactobacillus cells with pH curves produced by yeast and by lactobacillus separately (.03 molar glucose used as substrate).

this amount of glucose as substrate the addition of yeast cells to the lactobacillus cells has prevented the lactobacillus from reaching as low a pH as it produced when alone.

In another experiment in which a different type of yeast was used the cells were mixed with lactobacillus cells in 1:3, 1:1, and 3:1 proportions (by volumes) and .01 molar glucose was used as a substrate. The results are shown in *fig. 2*. Here again the initial pH levels of the mixtures were intermediate between the pH levels of the micro-organisms alone, but after the first 60 minutes an interesting phenomenon occurred. Although the lactobacillus alone maintained the

lowest pH levels throughout the experiment, the suspensions containing yeast cells inverted their respective pH positions. The mixture containing the least yeast and most (1:3) lactobacillus finally produced the highest pH level, while the yeast alone produced the second lowest pH level. It is apparent that the lactobacillus and yeast have supplemented each other to increase the consumption of acids. Two other experiments with other yeasts and lactobacilli gave similar results.

Experiments with mixtures of streptococci with lactobacilli are shown in *figs. 3* and *4*. *Fig. 3* shows the effect of mixing a strain of alpha streptococci which

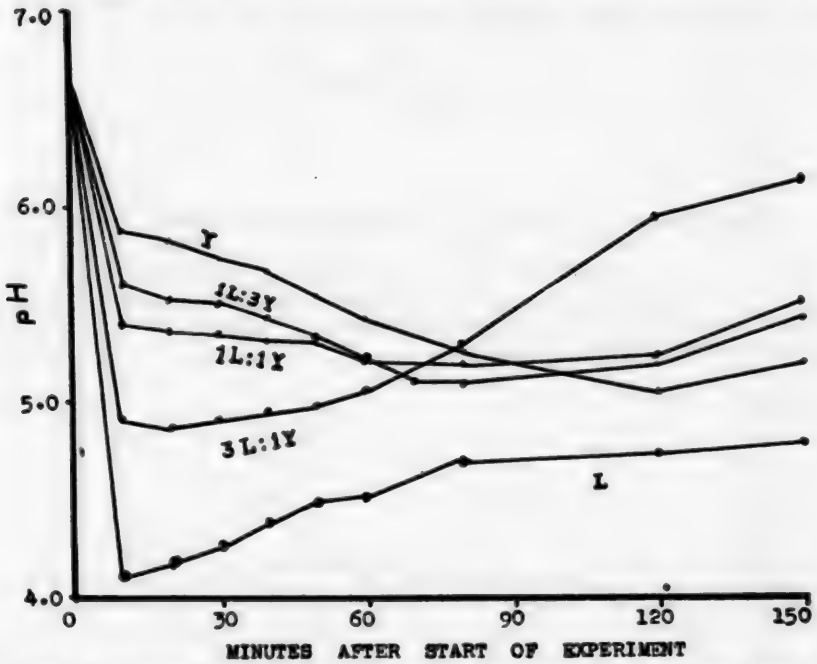


*Fig. 3.* Effect on pH curves of varying proportions of yeast and lactobacillus cells (01. molar glucose as substrate).

produced only slight pH changes by itself with lactobacillus. It can be seen that the mixgure of streptococci produced a relatively small difference from the pH curves produced by the lactobacillus alone and that this difference in pH was least at the end of the experiment.

*Fig. 4* shows pH curves of lactobacillus with another strain of alpha streptococci which by itself produced a rapid drop in pH with glucose. Here again, the difference in pH produced by the mixture of streptococci with lactobacilli was not very great, although the final pH difference was greater than in the preceding experiment. Apparently the greater ability of this second strain of alpha streptococci to produce acid was also accompanied by a greater ability to consume acid.

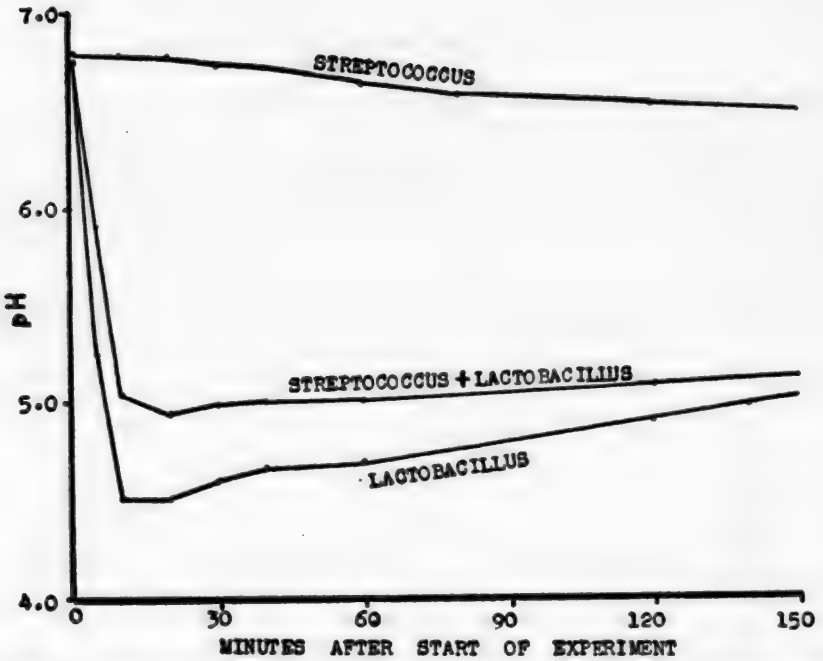


*Fig. 3.* Comparison of pH curves produced by a mixture of a weakly acidogenic streptococcus plus a lactobacillus with pH curves produced by streptococcus and lactobacillus separately (.01 molar glucose as substrate).

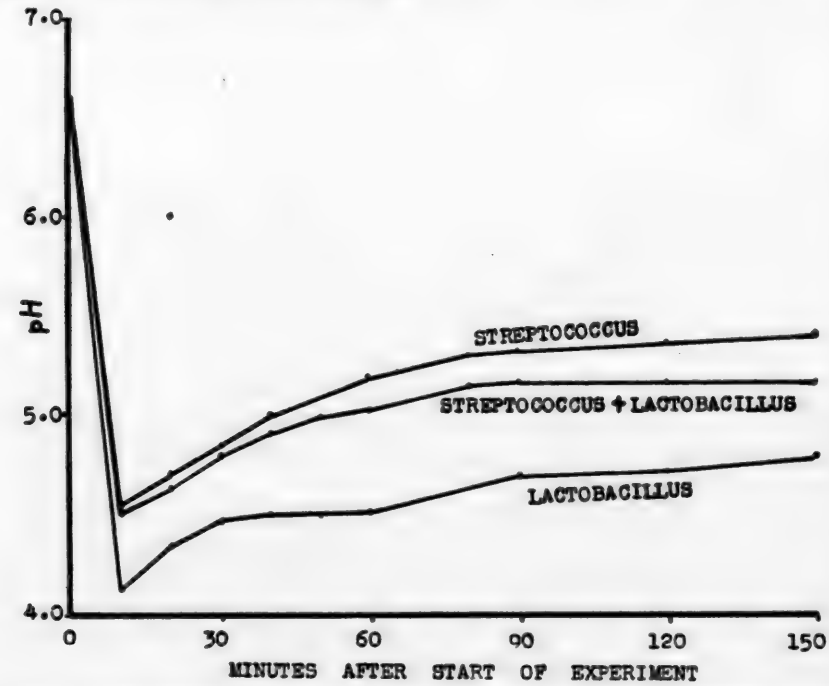


*Fig. 4.* Comparison of pH curves produced by a mixture of a strongly acidogenic streptococcus and lactobacillus with pH curves produced by streptococcus and lactobacillus separately (.01 molar glucose as substrate).

Thus, while the first strain of streptococci did not produce acid as rapidly as the second strain of streptococcus, nevertheless, it interfered the least with the action of the lactobacilli in producing low pH levels.

In caries of the dentin, the possibility exists that the micro-organisms in dentinal tubules may use the constituents of the fluids in the tubules ("dental

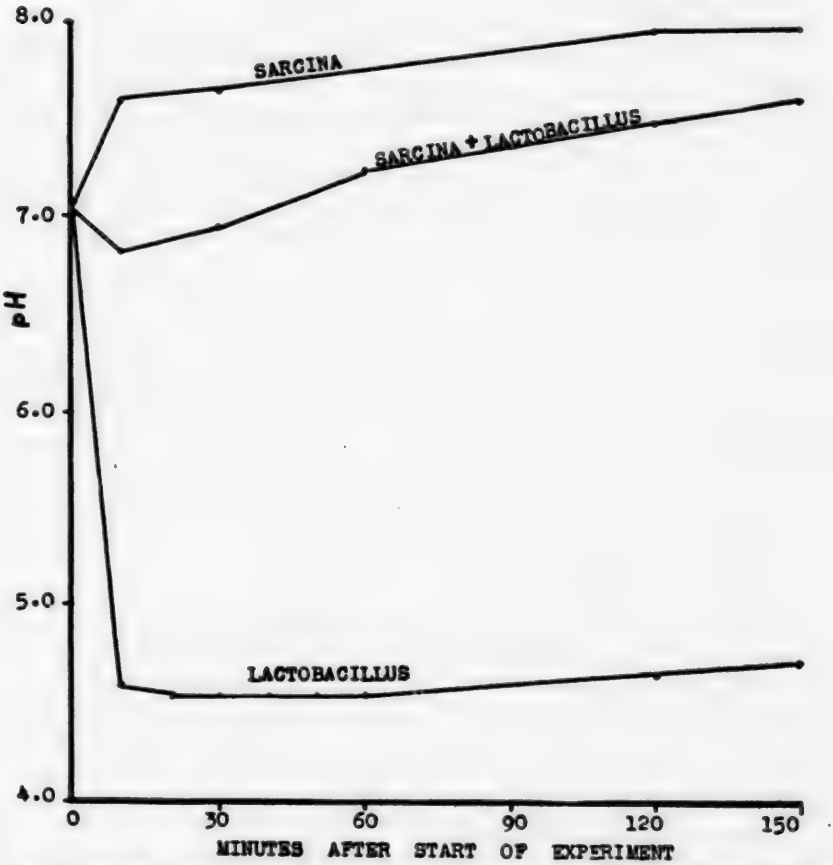


*Fig. 5.* Effect of mixing a urea splitting micro-organism, sarcina, with lactobacillus on pH curves produced in a buffer solution containing .0007 molar glucose and .003 molar urea.

lymph") as a substrate, as well as diffusible food substances. While the composition of "dental lymph" is unknown, it might contain glucose and urea in about the amounts as of other lymph, namely about .007 molar glucose and .003 molar urea. In order to illustrate the difference in pH curves which urea-splitting micro-organisms, non-urea-splitting micro-organisms, and mixtures of the 2 might produce in such a medium, experiments were run on a lactobacillus (non-

urea-splitter) and a sarcina (urea-splitter) and a mixture of the 2 in buffer containing .007 molar glucose and .003 molar urea. The results are shown in *fig. 5*. It can be seen that the sarcina alone showed a rise in pH, in combination with lactobacillus it showed a slight drop in pH followed by a prolonged rise, whereas, lactobacillus alone a marked drop in pH with only a very slow subsequent rise.

## DISCUSSION

The results of the experiments described in this paper appear to support the views of those investigators who hold that oral lactobacilli are the micro-organisms most likely to cause dental caries. The results seem to conflict with the interpretations which Bibby (2) and Fosdick (1) have given to the results of their own experiments in which they indicate that streptococci (2) and mixtures with yeasts (1) respectively are the micro-organisms most likely to cause caries. However, in such a complex phenomenon as dental caries, it is hazardous to carry over in vitro results to in vivo conclusions.

The chief characteristics of the experiments described here have been that high concentrations of microbic cells have been employed together with only limited amounts of substrate. If caries can be initiated on tooth surfaces only by dense populations of microbes (i.e. "plaques") then competition for substrates, as suggested in these experiments, would characterize the metabolic relationships between the different oral micro-organisms. On the other hand, if caries can be initiated on tooth surfaces contacted by relatively small bacterial populations, (i.e. by "saliva") in the absence of plaques then competition for a limited amount of substrate in a small area might not be important, and a synergystic effect might occur, as suggested by Fosdick.

Again if only a limited amount of fermentable carbohydrate remains in the mouth after eating food, then only those micro-organisms which are most efficient in producing and maintaining low pH levels from it (such as lactobacilli) would be able to decalcify teeth in vivo. Under these circumstances other micro-organisms mixed with lactobacilli might tend to prevent any decalcification from occurring. On the other hand, if an excess of fermentable carbohydrate can be maintained on tooth surfaces, then there seems no reason to doubt that most oral micro-organisms could produce decalcification of teeth, and might supplement each other in the caries process.

Finally, the presence in the mouth of substrates other than carbohydrates, may exert a considerable effect on pH levels, and certain of these substances may be of considerable importance in modifying the process of dental caries.

## SUMMARY

Experiments on pH were performed with mixtures of oral lactobacilli and some other oral micro-organisms at high microbic cell concentrations and with a limited amount of substrate. It was found that streptococci, yeasts, and sarcina prevented lactobacilli from reaching their lowest pH levels under these conditions.

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(2) BIBBY, B. G., VOLKER, J. F., AND VAN KESTEREN, M., Acid Production and Tooth Decalcification by Oral Bacteria. *J. D. Res.*, **21**: 61, February, 1942.

(3) (4) STEPHAN, R. M., AND HEMMENS, E. S., Studies of Changes in pH Produced by Pure Cultures or Oral Micro-organisms. I. Effects of Varying the Microbic Cell Concentration; II. Comparison of Different Micro-organisms and Different Substrates. *J. D. Res.*, **26**: 15, February, 1947.

# A METHOD FOR THE CHEMICAL MEASUREMENT OF ACTIVITY OF CARIES OF THE ENAMEL[1]

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The following experiments are believed to constitute chemical measurements of the rate of caries activity in enamel.

The experimental procedure (*fig. 1*) is an outline as follows: the passage of material from the oral cavity (E) to the material (C) on the enamel surface is prevented by means of a barrier during an interval of approximately 10 minutes and the concentration of total phosphate in C is measured twice during this period. It is assumed: (1) that the line of separation (B) of enamel from the overlying debris (C) is experimentally definite because of the hardness of the enamel surface in the early stages of caries and the softness of the debris; (2) that if the migration of phosphate from E to C is prevented, a rise in the total phosphate concentration in C can be caused only by a migration of phosphate from the enamel (A) to C; (3) that the rate of migration of phosphate from A to C is the rate of caries activity, as the only considerable source of phosphate in A is the apatite of which the enamel is composed.

## METHODS

*Material.* The areas studied were those underlying collections of fairly abundant, homogenous, white debris on tooth surfaces and have included etched areas in patients with extensive enamel caries and caries-free areas in normal mouths. The areas were selected several days before the experiment was performed and the debris allowed to accumulate without exposure to drugs, dentifrices or tooth brush. The areas chosen were the distal surfaces of the maxillary second incisors, the mesial or distal surfaces of the canine or the mesial surface of the first premolar.

*Plan of the Experiments.* At least an hour after the subject's last meal and after at least 5 minutes continuous closure of the mouth the previously selected area was exposed by opening the mouth and retracting the lips. Immediately thereafter the first sample of debris was removed with a Bates scaler, transferred to the loop (*fig. 2*) and stoppered into the weighing bottle; barrier and rolls were placed in position and the mouth closed. A second sample was taken from the same area at the selected time immediately after removing the barrier. In those experiments in which glucose was used 1 gram of powdered glucose was rapidly poured into the mouth 1 or 2 minutes prior to the first sampling, caused to dissolve in the saliva by movement of the tongue and brought into contact with the experimental area.

[1] Received for publication November 13, 1946

*Weighing of sample.* The weighing bottle was weighed on a micro balance immediately after collection of each sample; the weight of sample was calculated by the difference from a weighing of the vessel and loop without sample shortly before. The glass vessel was not removed from the balance case and, as the stopper was of cork, untoward temperature effects were prevented. The weighings were accurate to within 3 or 4 hundredths of a milligram with the usual weight (0.5 to 1.0 mgm.) of sample.

*Barrier.* After preliminary experiments with modeling compound and an acrylic resin shield prepared by Dr. R. M. Appleman, the most satisfactory barrier proved to be a layer of sticky wax (Kerr Dental Mfg. Co., Detroit),

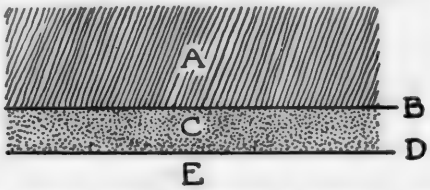


*Fig. 1.* A represents enamel; B, line of separation of enamel from C, mass of soft debris overlying enamel. D is line separating C from E, oral cavity.

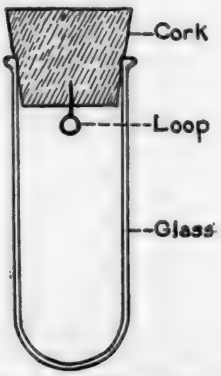


*Fig. 2.* Weighing vessel

This was warmed to about 40° C immediately before use and carefully adapted to the labial and lingual aspects of the teeth and alveolar ridge to a point well above the labial and lingual gingival margins extending the width of 2 teeth on either side of the experimental area and guarded by cotton rolls placed above and posterior to the labial flange. The purpose of the barrier is to prevent the access of saliva to the material being sampled during the experimental period.

*Measurement of total phosphate.* The method described was devised for these experiments after continued failure to attain, with the usual methods, a sufficient degree of accuracy, sensitivity and reliability.

Each platinum loop holding a sample was dropped cleanly to the bottom of a 16 x 150 mm. pyrex test tube previously tested for optical equivalence with the other tubes employed. Three blank tubes, each containing 0.5 ml. of

distilled water, and 3 standard tubes, each containing 2.5 micrograms of phosphorus in 0.5 ml. of water, were prepared simultaneously. To each tube was added 0.5 ml. of 1N perchloric acid and the tubes were heated in a transparent air bath at 150°C. for 30 minutes. If charring of the sample above the level of the liquid occurred the tube was shaken to wash the charred portion into the liquid. At the end of the digestion all tubes contained clear droplets. The digested samples were slightly yellow. All tubes were cooled and each received 0.50 ml. of water, 0.66 ml. of 4% sodium molybdate (Merck) and 3.30 ml. of 0.1% hydrazine sulfate (Merck) in 1N sulfuric acid. The reagents were thoroughly mixed after each addition. The tubes were placed in a water bath at 50°C. for 20 minutes and read in an Evelyn photoelectric colorimeter, provided with adapter for 16 x 150 mm. tubes, using filter 660. To each tube was then added 5 ml.

TABLE I

*Phosphate change in enamel*

| EXPERIMENT | SUBJECT | CARIES | GLUCOSE GIVEN | TIME INTERVAL | $C_1$ | $C_2$ | % CHANGE |
|---|---|---|---|---|---|---|---|
| | | | | *minutes* | | | |
| 1 | A | + | 0 | 4½ | 2.25 | 1.87 | −15 |
| 2 | A | + | + | 4½ | 2.25 | 3.75 | +67 |
| 3 | A | + | + | 9 | 6.75 | 14.6 | +117 |
| 4 | A | + | + | 9 | 3.34 | 3.70 | +11 |
| 5 | A | + | + | 9 | 4.19 | 3.65 | −13 |
| 6 | A | + | + | 9 | 4.50 | 7.12 | +58 |
| 7 | B | 0 | + | 13 | 3.64 | 3.75 | +3 |
| 8 | B | 0 | + | 10 | 3.00 | 2.00 | −33 |
| 9 | B | 0 | + | 10 | 3.75 | 3.38 | −10 |
| 10 | B | 0 | + | 10 | 3.75 | 2.62 | −30 |
| 11 | B | 0 | + | 10 | 4.12 | 3.25 | −20 |

Subject A, a 12 year old girl with very extensive caries.

Subject B, a 45 year old laboratory worker.

$C_1$ and $C_2$ are the concentration of total phosphate in micrograms per milligram of sample 1 and sample 2 respectively. The time interval is that between the taking of sample 1 and sample 2.

of normal butanol, and nitrogen was bubbled through the tubes for 2 minutes as rapidly as could be done without loss. The layers were allowed to separate and the aqueous layer was again read in the colorimeter. The loss in optical density on extraction was calculated for each tube and the average value of this loss for the blank tubes was subtracted from the values for the other tubes. The phosphate of the samples was calculated by interpolation. All glassware was cleaned with chromic acid, sulfuric acid and rinsed well with distilled water and finally with 95% ethanol between determinations. The platinum loops were similarly treated. For wiping pipettes between transfers tissue paper squares which were almost free of phosphate were used. The probable error of the method with 0.5 to 1.0 mgm. samples was 3.6%.[2]

[2] The development of the chemical methods was greatly facilitated by the technical assistance of Miss Rebecca Woodson.

## DISCUSSION

Table I includes only the results of experiments performed with exactly the technique described and includes all such experiments with no visible failure of the barrier to exclude saliva or other technical faults. Leakage of saliva into the experimental area after the removal of the first sample lowers the concentration of phosphate in the second sample, as the concentration of phosphate in saliva is only about a tenth of that in the soft material on the enamel surface. The results of some hundred experiments performed before the exact technique described above was developed confirm the findings in Table I.

As the results in the table indicate (Experiments 2, 3, 6) significant rises in phosphate concentration occur over carious areas exposed to glucose and not (Experiments 7, 8, 9, 10, 11) over non-carious areas. The falls in phosphate concentration observed in Experiments 8 and 10 are probably due to unobserved leakage of saliva from the gingival margins into the experimental area and if leakages of equal magnitude occurred in the remaining experiments they could have concealed barely significant rises in Experiment 1, 5, 7 and 9 and lowered the rises observed in Experiments 2, 3 and 6 from higher values; so that, while low degrees of activity may, with the present technique, escape measurement the method measures high degrees of activity with an accuracy of probably about 30%.

These experiments were undertaken after reviewing a number of serial x-ray films of advancing carious lesions, from which it was roughly estimated that the loss of apatite from carious area of the enamel is of the order of magnitude of a milligram a month or 40 micrograms of phosphorus per average 10 minute interval. Assuming, in experiment 2, that there are 4 milligrams of debris overlying the carious area, the total passage of phosphorus from enamel to debris would be $4 \times (3.75 - 2.25)$ or 6 micrograms in $4\frac{1}{2}$ minutes. The rate found experimentally is thus of the order of magnitude predicted.

The significance of the method is believed to lie in the fact that it yields conclusive results in short term experiments with the carious lesion in a relatively undisturbed state.

## SUMMARY

It has been shown possible to measure the rate of caries activity in enamel in accessible areas by chemical means.

# CARIOUS LESIONS IN COTTON RAT MOLARS[1]

## I. The Role of Mechanical Factors Studied by the Extraction of Antagonistic Molars

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The cotton rat (*Sigmondon hispidus hispidus*) has been found by Shaw, Schweigert, McIntire, Elvehjem and Phillips (1) to be readily susceptible to the development of carious lesions in the sulci of the molars, independent of the particle size of the purified diets on which the animals were maintained. Since Hoppert, Webber and Canniff (2), Sognnaes (3) and Ginn (4) have shown that mechanical factors were of considerable importance in the production of certain types of carious lesions in the white rat it was of fundamental importance to determine if mechanical factors affected the development of the experimental carious lesions of cotton rats. In this experiment the mechanical effects of mastication were altered by the extraction of the maxillary molars of 1 quadrant. The maxillary molars were selected for extraction because of the higher incidence of carious lesions observed in the mandibular molars by Shaw, Schweigert, McIntire, Elvehjem and Phillips (1). At the termination of the experiment the incidence and extent of carious lesions in the mandibular molars which had opposing molars could be compared to the incidence and extent of lesions in those mandibular molars which had no opposing molars.

### EXPERIMENTAL

The 25 cotton rats used in these studies were obtained from our stock colony at approximately 2 weeks of age. The right maxillary molars were removed at that time while the animals were under ether anesthesia. The first and second molars were extracted with the aid of a pair of orthodontic pliers, the jaws of which had been ground to clasp the crowns. Since the third molar has not erupted into the oral cavity at this age, it was necessary to probe into the alveolar ridge distally and slightly buccally to the second molar with an explorer until the unerupted crown could be contacted and forced into the oral cavity. In a few cases the third molar crown could not be located at this age and therefore was left until after it had begun to erupt when it could be extracted in similar fashion to the first and second molars.

[1] This project was supported in part by the Sugar Research Foundation, Inc., New York. Received for publication October 28, 1946.

The author is indebted to Merck and Company, Rahway, New Jersey for the synthetic vitamins and to the Wilson Laboratories, Chicago, Illinois, for 1:20 liver concentrate powder and whole liver substance.

For a period of 14 weeks after the extraction of the right maxillary molars, these 25 cotton rats were fed a purified ration very comparable to that used by Shaw, Schweigert, McIntire, Elvehjem and Phillips (1). The percentage composition of the diet used in the present experiment was: sucrose 67, casein 24, salts 4, and corn oil 5. The crystalline vitamin B complex vitamins had been added to the casein in sufficient amounts that each 100 g. of the diet contained:

TABLE I

*The effect of extraction of the right maxillary molars upon the incidence and extent of carious lesions in the right mandibular molars of the cotton rat*

| ANIMAL NO. | SEX | NO. OF CARIOUS LESIONS IN | | EXTENT OF CARIOUS LESIONS IN | |
|---|---|---|---|---|---|
| | | Mandibular left molars | Mandibular right molars | Mandibular left molars | Mandibular right molars |
| 1 | M | 10 | 10 | 45+ | 41+ |
| 2 | F | 10 | 8 | 34+ | 23+ |
| 3 | M | 9 | 7 | 30+ | 22+ |
| 4 | M | 8 | 7 | 33+ | 25+ |
| 5 | F | 8 | 6 | 26+ | 18+ |
| 6 | F | 8 | 6 | 23+ | 14+ |
| 7 | M | 8 | 5 | 22+ | 11+ |
| 8 | F | 8 | 7 | 25+ | 17+ |
| 9 | F | 8 | 7 | 31+ | 21+ |
| 10 | M | 8 | 7 | 28+ | 19+ |
| 11 | F | 7 | 6 | 23+ | 18+ |
| 12 | F | 7 | 7 | 21+ | 17+ |
| 13 | M | 6 | 4 | 7+ | 5+ |
| 14 | M | 6 | 6 | 20+ | 14+ |
| 15 | M | 6 | 5 | 14+ | 10+ |
| 16 | M | 5 | 4 | 12+ | 8+ |
| 17 | F | 5 | 3 | 8+ | 5+ |
| 18 | M | 5 | 1 | 12+ | 3+ |
| 19 | F | 5 | 6 | 17+ | 19+ |
| 20 | M | 5 | 4 | 11+ | 8+ |
| 21 | F | 4 | 4 | 6+ | 7+ |
| 22 | F | 4 | 2 | 5+ | 2+ |
| 23 | M | 4 | 0 | 6+ | 0 |
| 24 | M | 3 | 2 | 13+ | 5+ |
| 25 | M | 3 | 2 | 7+ | 2+ |
| Average..... | | 6.4 ±0.4 | 5.0 ±0.5 | (19 ±2)+ | (13 ±2)+ |
| Difference..... | | 1.4 ±0.6 | | (6 ±3)+ | |

thiamine hydrochloride 350 ug., riboflavin 350 ug., pyridoxine hydrochloride 350 ug., nicotinic acid 2.5 mg., calcium pantothenate 2.0 mg., choline chloride 100 mg., inositol 100 mg., and para-amino benzoic acid 30 mg. The fat-soluble vitamins had been added to the corn oil in sufficient quantities that each 100 g. of the diet contained: beta-carotene 1.1 mg., irradiated ergosterol 300 I. U., 2-methyl-1, 4-naphthoquinone 0.6 mg., and alpha-tocopherol 5.0 mg. To each 100 g. of this diet were added 2.0 g. of 1:20 liver concentrate powder and 2.0 g.

of whole liver substance. The salt mixture described by Phillips and Hart (5) was modified by increasing the sodium, chloride, copper and zinc contents, adding cobalt, and adjusting the acid-base balance so that it would be approximately neutral when the phosphorus carried by 24% casein was considered. The salt mixture used in this experiment was made by mixing the following amounts of reagent grade chemicals: calcium carbonate 300 g., anhydrous dibasic potassium phosphate 470 g., anhydrous dibasic calcium phosphate 680 g., dried magnesium sulfate 100 g., sodium chloride 670 g., potassium chloride 115 g., ferric citrate 55 g., potassium iodide 1.6 g., manganese sulfate monohydrate 9 g., anhydrous zinc chloride 4 g., anhydrous cupric sulfate 2.4 g., and cobalt chloride 0.2 g.

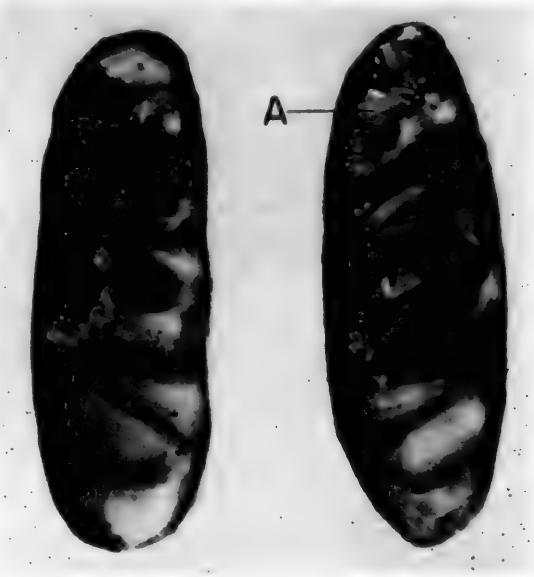


*Fig. 1.* The occlusal surfaces of left and right mandibular molars of cotton rat #1 are shown. Similarity in incidence and extent of carious lesions in 2 quadrants can be seen. Cusp labeled A is only one in molars which had no antagonistic molars, which was less affected by carious process than those of molars with opposing molars.

At the end of 14 weeks on experiment, these cotton rats were sacrificed and their heads fixed in 95% alcohol for 24 to 48 hours. The observations on the carious lesions of the molar teeth were made by grinding and observing several successive planes parallel to the occlusal surface. The criteria used for the evaluation of the carious lesions have been described previously by Shaw, Schweigert, McIntire, Elvehjem and Phillips (1).

## RESULTS

The effect of extraction of the right maxillary molars upon the incidence and extent of carious lesions in the mandibular molars of the cotton rats studied is

shown in Table I. The occlusal surfaces of the mandibular molars of cotton rat #1 are shown in *fig.* 1. In 21 of the animals, the number of carious lesions in the molars of the left mandibular quadrant, i.e., the molars which were in occlusion, was slightly higher than the number in the molars of the right mandibular quadrant which had no antagonistic molars. In 3 animals, there were the same number of lesions in the molars of each mandible. In only 1 animal, there was 1 lesion more in the molars of the right than in those of the left mandibular quadrant. The average number of carious lesions in the mandibular molars with antagonistic molars was $6.4 \pm 0.4$, in comparison with $5.0 \pm 0.5$ for the molars with no antagonistic molars. The difference, $1.4 \pm 0.6$, is just barely significant. In each case the number of carious lesions in the molars of the left mandible was characteristic of the susceptibility of the strain of cotton rats which the particular animal represented.

The extent of tooth substance involved in the carious process was slightly greater in the left mandibular molars of 23 cotton rats than in their right mandibular molars which had no antagonistic molars. The reverse was true in the 2 remaining animals. The average extent of carious lesions in the mandibular molars with opposing molars was $(19 \pm 2) +$, in contrast to $(13 \pm 2) +$ in the mandibular molars with no opposing molars. The difference, $(6 \pm 3) +$, is borderline in significance.

The rate of growth of these cotton rats was not adversely affected by the reduction in mastication resulting from the extraction of the right maxillary molars.

The amount of attrition on the occlusal surfaces of the left mandibular molars was similar to the amount observed in cotton rats in which all maxillary molars were present. There was no evidence of attrition on the occlusal surfaces of the right mandibular molars which had no antagonistic molars.

## DISCUSSION

Hoppert, Webber and Canniff (2) and others have shown that carious lesions occur in white rats maintained on a diet composed of coarse, yellow corn meal, whole milk powder, linseed meal, alfalfa meal and sodium chloride. If the corn meal was ground fine enough to pass through a 60 mesh sieve, no carious lesions developed in the sulci of the molars. In the cotton rat, particle size has been observed to be of lesser importance than in the white rat by Shaw, Schweigert, McIntire, Elvehjem and Phillips (1).

Sognnaes (3) studied the effect in the white rat of removing all maxillary molars or those on the right side upon the incidence of carious lesions in the mandibular molars. When all maxillary molars were removed only a few carious lesions were observed in either the right or left mandibular molars. When the right maxillary molars were removed, few carious lesions were observed in the right mandibular molars but a moderately high incidence of carious lesions was observed in the left mandibular molars. However, when all maxillary molars were present, there was a bilateral distribution of the carious lesions and the incidence of carious lesions in either jaw was about double the incidence of lesions in the left mandibular molars of the rats from which the right maxillary molars had been removed.

In a similar experiment, Ginn (4) removed the right maxillary molars of one group of rats after 100 days on a caries-producing diet and then maintained the rats on the same diet for an additional 60 days. A second group of rats was sacrificed after 100 days on the caries-producing experimental regimen. A close correlation in the incidence of carious lesions and areas affected was observed in the mandibular molar teeth of either side of the rats in group II and in the mandibular molar teeth which had no antagonistic molars in group I. However, the left mandibular molars of group I where the opposing molars were present throughout the 160 days had a much higher incidence of carious lesions. The data of Sognnaes (3) and Ginn (4) indicate the importance of the environmental factors arising from the mastication of coarse particles in the initiation of carious lesions in the sulci of the white rat. In addition, fractures have been found to be the first step in the pathogenesis of these rat molar lesions (Kearney, Sognnaes and Bibby (6)) and others. In contrast, the mechanical effect of mastication and enamel fractures appear to have relatively little effect on the incidence and extent of carious lesions in the cotton rats fed purified diets. The elimination of this complicating factor is of fundamental importance for further experimental investigation of the initiation and progress of carious lesions in the cotton rat and for future studies on possible relations between experimental carious lesions in cotton rats and those lesions occurring in man.

## SUMMARY

In comparison to the white rat, the extraction of maxillary molars in the cotton rat has relatively little effect on the initiation and progress of carious lesions in the molar teeth which have not been subjected to the stress of mastication. There is a barely significant reduction in the incidence and extent of tooth decay in the sulci of the mandibular molars which had no opposing molars.

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# THE ILLUSORY SHEATH OF NEUMANN IN DENTIN[1,2]

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## INTRODUCTION

The sheath of Neumann is described as a lining of the dentinal tubule, more resistant to acids and alkalies than the surrounding matrix. Staining decalcified transverse sections of dentin causes the circumference of each tubule to appear more marked, giving the impression of a lining or sheath. The mass of delicate tubes or fibers remaining after treating dentin with strong mineral acids (*fig. 1*) presents further confirmatory evidence of Neumann's sheath. Orban (1) described this sheath as "comparable to the so-called capsule of the lacunae in bone." Noyes, Schour and Noyes (2) elaborated on the discussion active since Neumann first described this structure in 1936. They stated in part: "Recent investigations indicate that Neumann's sheath does not exist as a separate structure, but, that the matrix which forms the immediate wall of the tubules is more highly refractive and appears as a varying halo around the dentinal tubules. These structures are therefore in no sense a sheath surrounding the dentinal fibril. . . .Apparently the sheaths of Neumann are composed of a material similar to that forming elastic connective tissue fibers and known as elastin. . . . Some authors have suggested that the great elasticity of the dentin was largely due to the presence of this substance."

In spite of the almost general acceptance of the existence of Neumann's sheath, a number of investigators hold the opposite view. Among these,

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[2] Editor's note: The original manuscript for this paper was submitted by the author on October 19, 1945. The manuscript was sent to each of the five consultants for comment and the comments and reply were inserted into the manuscript by the author. This is a discussion by mail and has been suggested by Dr. Bodecker as a technique for papers on controversial subjects.

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R. R. Andrews (3), as far back as the end of the century, maintained its non-existence and more recently Meyer and Churchill (4) made a similar statement. The latter discuss the subject at length. Their conclusions concerning the tubular wall in dentin matrix follow: "The dentinal tubules are simple recesses in the ground substance of the dentin and contain the dentinal fibrils. These processes of the odontoblasts may be isolated by mechanical and chemical means. A special wall which is designated as a sheath does not exist."

Churchill (5) ingeniously showed why the circumference of dentinal tubules stain darker than the surrounding matrix by piercing a thin sheet of celloidin with needle holes and then staining the sheet. The dye on the surface of

*Fig. 1.* Residue (fibrous or tubular?) after macertating dentin in 50% nitric acid. One lateral branch (LB) is visible (Orig. mag. 450×).

the celloidin is barely visible, but the peripheries of the holes appear deeply stained (*fig. 2*). The eye of the observer views the holes from above and sees stain along the line A–B. As the stain is much thicker (A–B) than that on the combined upper and lower surfaces of the sheet, the circumferences of the holes appear darker. Churchill held that this explains the semblance of sheaths surrounding dentinal tubules. He also claimed that the structures retained after macerating dentin in strong nitric acid are not sheaths lining the tubules, but are processes of odontoblasts, i.e., the dentinal fibrils.

In order to evaluate Churchill's hypothesis, it is necessary to examine the dentinal fibril in greater detail. Tomes' fiber is described as a protoplasmic process originating from the odontoblast. More recently (6, 7), it was described

as a tube and not as a fiber. This new conception (8) was demonstrated by washing small fragments of fresh dentin in pyridin under vacuum, thus removing the tissue fluid (dental lymph) within the odontoblastic processes. Transverse sections of tubules in this specially treated dentin showed the dentinal fibrils

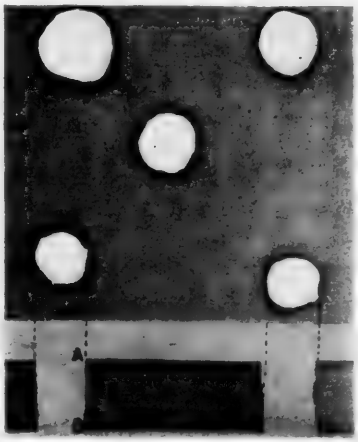

*Fig. 2.* Diagram of sheet of celloidin pierced with holes shows that staining of dentin sections gives false impression of sheath lining dentinal tubules. (After Churchill)

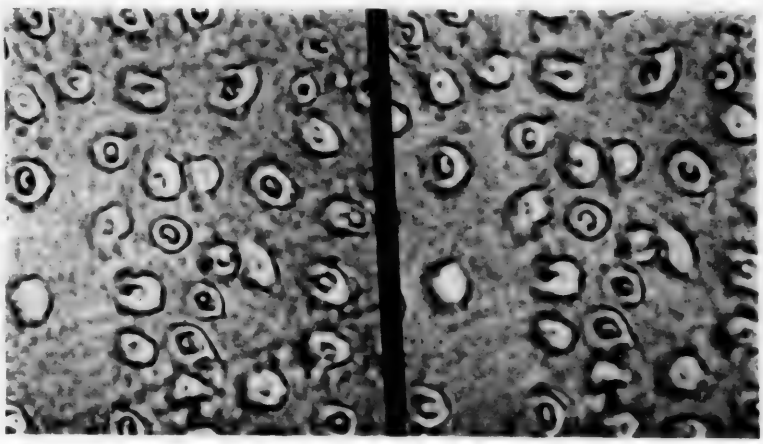

*Fig. 3.* Photomicrograph with ultraviolet light of pyridin treated sheep dentin shows tubular character of dentinal fiber in transverse section; two views of same area with 1 micra change of focus.

as rings instead of discs (*fig. 3*). Even routinely prepared dentin occasionally shows a similar picture on careful examination because dentinal fibrils occasionally contain vacuoles (*fig. 4V*). When the line of section (S) passes through one of these, the fiber appears as a tube cut transversely. Little is known as

yet concerning these vacuoles, but it is believed that they result from age changes or external irritation of the dentin (9, 10).

Knowledge of these details is necessary to the comprehension of Churchill's contention that the structures remaining after dentin maceration with strong mineral acids are dentinal fibrils and *not* tubular structures. He based his claim that Neumann's sheath does not exist on this very point. Most investigators, on the other hand, depict the isolated structures as tubes and thus subscribe to Neumann's views. But if the processes of odontoblasts prove to be tubular, then these might have been regarded erroneously as sheaths lining the dentinal tubules (Neumann's sheath).

One further observation which throws some light on the character of dentinal fibrils must be mentioned. W. Lefkowitz, an associate of the author, called attention to a particular section of dentin in which the dentinal fibers were visible. The striking part of this observation was that the pulp had been extirpated more than five years before the removal of the tooth. The long continued survival

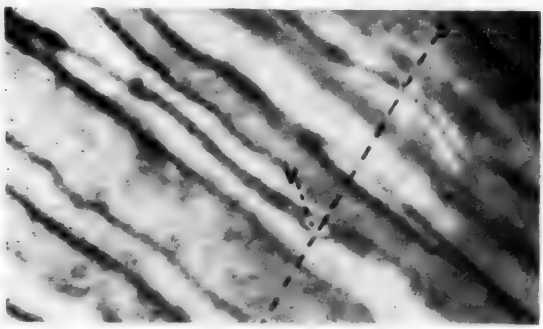

*Fig. 4.* Longitudinal section of untreated dentin showing vacuole (V) in dentinal fiber. If section had passed along indicated line (S) fiber would have appeared as tube. Heidenhain ferric haematoxylin (Orig. mag. 4500×).

of dentinal fibers under these conditions suggests their great resistance to decomposition. Hence further study of the structure is indicated.

The purpose of this investigation is to determine whether or not dentinal fibers:

1. Are responsible for the conception of Neumann's sheath surrounding the dentinal tubules
2. Are tubular and persist after maceration of dentin
3. Are composed of a more resistant material than protoplasm (cytoplasm).

## METHODS

The oldest method of demonstrating Neumann's sheath was to macerate pieces of dentin, place the resultant residue on a slide with or without previous staining and examine under the microscope. This technique showed fine tubes always in longitudinal aspect because of the length of the tubes. If, on the other hand, thinly ground sections of dentin are used for maceration,

then a considerable number of very short tubes show up under the microscope in transverse and tangential sections. Therefore, mostly thin sections were employed in preference to large pieces of dentin.

The reagents used in the past to separate the structural elements of the dentin were strong acids and alkalies. In this investigation high heat had been used in addition in order to eliminate the protoplasmic dentinal fiber claimed by Churchill as the illusory Neumann's sheath.

Three different methods were used:

1. The technique employed by Kitchin (11) for decalcification under the coverglass was used in these experiments. This consists of placing on a microscope slide a ring of petroleum jelly about 3–5 mm. high and 20 mm. in diameter In the center was placed a very thin ground section of dentin (50–70 microns), immerced in 50% nitric acid and covered with a coverglass. The petroleum jelly forms a fairly satisfactory seal so that with care specimens may be preserved for some time and with little danger of the microscope being harmed by the acid. Successive photomicrographs were taken with a vertical camera showing the gradual destruction of the dentin matrix ranging from 24–48 hours.

2. Axial ground sections of dentin approximately 130 microns thick were placed in individual platinum foil envelopes. Access of air to the sections was fairly free as the envelopes were not tightly sealed. The specimens were then heated in an electric furnace up to 1100°C. within a period of eleven minutes and kept for two minutes at this temperature. After transferring the fragments from the platinum envelope to a microscope slide as described above, 25% nitric acid was added. Photomicrographs were then taken at intervals during decalcification, which appeared to be complete in 24–30 hours. The remnant of the macerated dentin was unchanged at the end of 3 weeks.

3. Entire teeth or axially cut slabs 3–5 mm. in thickness were over-decalcified in 5% nitric acid. Third molars of persons ranging in ages from 20–23 years were usually used as these decalcify much more rapidly than older teeth. The material was then subjected to the routine treatment of washing, neutralization of acid, and dehydration. Because the dentin was partially macerated it was possible to use paraffin as the embedding medium and thus obtain exceedingly thin sections (4–5 microns). These were stained with Delafield haematoxylin-eosin, von Gieson, Mallory or Bielschowsky methods.

## FINDINGS

All observations made on specimens prepared by the first and second methods were similar to those reported by previous investigators with one exception, i.e. transverse sections of tubules were visible under the microscope.

*Method 1.* The clearest photomicrographs are made when the tubes or fibers are completely isolated from the matrix. *Fig. 5* shows 2 groups held together by only a little matrix (x). *Fig. 6* demonstrates 2 masses of partly macerated dentin and 2 completely isolated tubes or fibers having a wavy appearance (SC). These resemble the secondary curvature of the dentinal tubules and thus suggest that they are Neumann's sheaths.

*Method 2*. The incineration of dentin resulted in a series of miniature explosions of the sections but loss of the fragments was prevented by previous enclosure in platinum foil envelopes.

*Fig. 5*. Partially macerated dentin showing 2 groups of tubes or fibers (×) held together by small amount of matrix (Orig. mag. 1000×).

*Fig. 6*. Same as fig. 5. Two structures suggest secondary curvature (SC) of dentinal tubules (Orig. mag. 1000×).

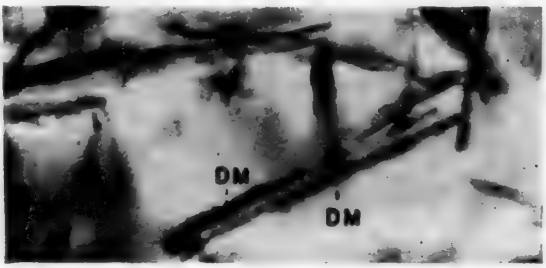


*Fig. 7*. Residue of partially incinerated and macerated, thin section of dentin giving appearance of tubules. Dentin matrix (DM) appears clinging to one tubule (Orig. mag. 3000×).

The nitric acid treatment caused by methods one and two, came to a standstill after about 6 hours. Delicate tubes could still be seen (*figs. 7, 8*) in specimens treated for 3 weeks (method 2). A lateral branch (8LB) is suggestive of the dentinal fibril. However, 2 tubes in *figs. 7* and *8*, at "DM" show

*Fig. 8.* Same as *fig. 7*, LB = lateral branch; DM = dentin matrix.

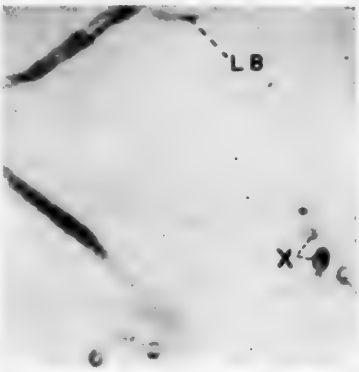


*Fig. 9.* Same as *figs. 7* and *8* showing transverse (×) and tangential sections proving that isolated structures are tubes; LB = lateral branch of dentinal fiber (Orig. mag. 3000×)

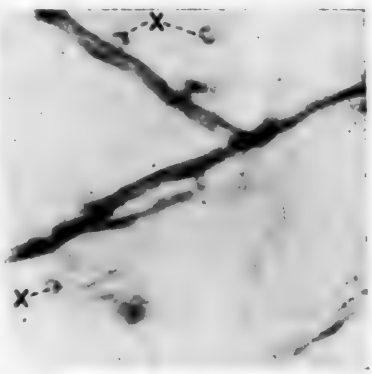


*Fig. 10.* Same as *fig. 9* showing transverse sections of dentinal fibers (×) (Orig. mag. 3000×).

thickening of their walls which appear to be remnants of the immediately adjacent matrix not destroyed by maceration. All structures seen in these 2 figures have the appearance of tubes; this interpretation is confirmed by *figs.*

*9* and *10*. Here a number of transverse sections may be noted as definite rings, demonstrating the tubular character of the isolated structures.

The specimens obtained by the use of methods one and two are very similar in appearance. The only difference seems to be a greater brittleness of the isolated tubes in specimens prepared by heat and acid treatment (Method 2).

*Fig. 11.* Section of a tooth slab over-decalcified and stained according to Mallory. Neumann's "sheath" destroyed, dentinal fibers (F) retained in longitudinal section. Lateral branch (LB) and vacuoles (V) in fiber (Orig. mag. 4500×).

*Fig. 12.* Somewhat more advanced destruction than in *fig. 11*. Some matrix fibrils (MF) running at right angles with dentinal fibers (F). (Orig. mag. 4500×)

Slight pressure on the coverglass resulted in breaking of the tubes into smaller lengths.

Methods one and two in which complete maceration of the dentin is employed, be it a large piece or a thin section, have a common fault in that the relationship of all dentin structures is lost. Hence it is impossible to observe whether the

isolated tubes are sheaths of the dentinal tubules or dentinal fibers in tubular form.

Method 3. The partial maceration of whole teeth or tooth slabs retains all structures in their normal relationship. Specimens prepared by this method show a persistence of the dentinal fibers while the lining of the dentinal tubules

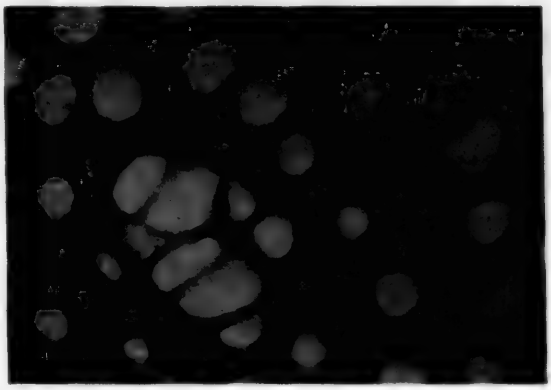

*Fig. 13.* Same specimen as in *fig. 11* sectioned transversely; tubules enlarged through destruction of their walls causing fibers to drop out. Neumann's "sheath" has disappeared. (Orig. mag. 4500×)

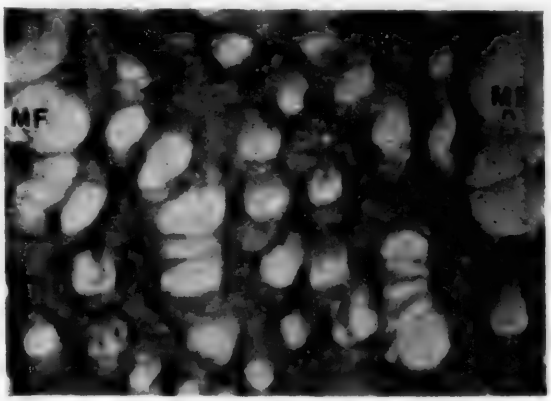


*Fig. 14.* Same as *fig. 13.* Coalescing tubules separated by matrix fibrils (MF) (Orig. mag. 4500×).

is no more resistant to acid than is the rest of the matrix. *Figs. 11* and *12* show the progressive disintegration of the matrix composing the walls of the tubules while the dentinal fibers remain intact; but there is no sign of a sheath. In *figs. 11* and *12*, some tubules in longitudinal section are slightly enlarged; others have become confluent. The progressive destruction of the matrix surrounding the dentinal tubules can be observed even more convincingly

in transverse sections (*figs. 13* and *14*) in which a number of tubules have coalesced. Dentinal fibers usually drop out of thin, transverse sections of tubules, probably due to the fact that fibers have no organic connection with the matrix unless lateral branches are present.

## DISCUSSION

Two conflicting observations were made on specimens prepared by methods one and two. One, the fact that undissolved matrix adhered to the isolated tubes (*figs. 7* and *8*) and second, that lateral branches were observed after maceration of the dentin. The former appeared to reaffirm the presence of a dentinal tubular lining (Neumann's sheath); the latter suggested that the dentinal fiber survives acid maceration. By and large, it seemed at the time methods one and two were used, that Neumann's sheath was present because it appeared incredible that the protoplasmic process of the odontoblast (cytoplasm) could survive high heat. The acceptance of this hypothesis would have involved the corollary that the lining of the dentinal tubule (Neumann's sheath) would extend into its lateral branches.

Meyer and Churchill (4) maintained that structures isolated after maceration of dentin by acid are fibers and that their tube-like appearance is due solely to refraction caused by incorrect focussing of the microscope. There is no doubt that this illusion can occur if only longitudinal aspects of tubes are observed. But transverse sections of the "fibers" show them as clearly defined rings (*figs. 9* and *10*) thus demonstrating that the structures isolated from macerated dentin are tubular. Because the identity of the isolated tubes or fibers still remained doubtful, some years ago the author carried out the second set of experiments in which dentin was incinerated at a temperature of 1100°C. This procedure was thought to destroy the protoplasmic dentinal fibers and thus eliminate the possibility of regarding them as linings of the dentinal tubules (Neumann's sheath).

The results of this experiment were similar to those observed by macerating non-incinerated dentin, i.e., a mass of delicate tubules still remained. The fact that these survived the high heat *and* the 25% nitric acid treatment seemed evidence that the isolated structures art not the cytoplasmic processes of the odontoblasts, but must be exceptionally resistant matrix linings of the dentinal tubules. The presence of Neumann's sheath therefore seemed to be reaffirmed, and as nothing new seemed to have been added to our knowledge of the dentin structure, no report was made on the above observations.

The more recently observed fact that dentinal fibers survived 5 years after the removal of the pulp created renewed interest in the subject. The third experiment cast an entirely different light on the problem and the observations made on specimens prepared by the second method. When entire teeth or tooth slabs were only partially macerated, the dentinal fibers remained, while the linings of the dentinal tubules disappeared completely.

This investigation also throws an interesting light on the nature of the dentinal fiber. Present experiments suggest that it is unlikely that the odontoblastic

"fiber" is composed of cytoplasm, hence further experiments are in progress with the hope of answering these questions.

## CONCLUSIONS

The structures remaining after dentin maceration are tubular; these do not represent Neumann's sheath, but are the tubular processes of the odontoblasts, the dentinal "fiber."

No change was made in the manuscript by the author after submission to the consultants with the exception of the substitution of the word "fiber" for "fibril".

## DISCUSSION BY CONSULTANTS

DR. CHEYNE: "In reading over the paper I am skeptical as to the possibility of eliminating the protoplasmic dentinal fiber by incineration. It seems more likely the fiber would be ashed, thus leaving a shadow outline of its former self. Immediate coagulation of the protoplasm of the fibers by acids or heat can cause a peripheral "tubing effect." This could be interpreted as remaining "sheath." If this is not true how is one to conclude that a Neumann's sheath can be present? Obviously, if it is an organic structure this would disappear then with ashing; if inorganic, it would be dissolved by acid.

"It appears this research is essentially concerned with methods and demonstrates clearly how opposite views can become established depending upon the method used as proof. From the evidence presented I am quite in agreement with the author who concludes that the structures designed as Neumann's sheath are probably the remains of odontoblastic fibers. We need to know more about the primary deposition of salts, especially on the peripheral surface such as the tube presents before we can definitely settle the question of Neumann's sheath."

DR. CHURCHILL: "Far away from the laboratories of active research in the realm of dentistry and without access to libraries or my own writings I must confine myself necessarily to only a few remarks.

"If I remember well, I never denied outright the presence of a sheath of Neumann, but concluded in my essay referred to in Bodecker's introduction that the existence of such a structure had never been satisfactorily proven.

"It seems to me that many of us who have concerned ourselves for years with the study of the minute anatomy of the teeth put too much faith in what we see without due regard to the circumstances which condition seeing. Furthermore, it appears that in our eagerness and enthusiasm to spread light on thus far unexplainable phenomena our minds tend to outrun proper reasoning principles. We are given to think that the teeth are unique structures, but so are the nails and so is the gastric mucosa, if we come to think of it, all structures are unique which perform a function different from all structures. However they all have a common ancestry and therefore show certain family traits. In the different groups of principal tissues these signs of kinship are more easily discernible, thus we might consider dentin as a type of bone. Irregular dentin cannot even always be distinguished from certain forms of bone. The morphologic principle of the cells which become enclaved in irregular dentin is the same as that of osteocytes and odontoblasts; they both consist of nucleoplasm and cytoplasm, the latter forming extensions which occupy the canaliculi of bone and the tubuli in the dentin (the dentinal fibers).

"Now this may not be true, but thus far we always have accepted it as true, if it is not true we want proof of it. We can not hypothesize that dentinal fibers are hollow tubes filled with lymph for a hypothesis is a seemingly true statement and this statement does not seem true but unique.

"For many years, Bodecker sincerely believed that the dentinal fibers he obtained after maceration of the dentin were the sheaths of Neumann and not the dentinal fibers. After convincing himself of the contrary he was guided by his intellectual honesty to publish his change of view. However, he still clings to the tubular nature of lymph containing extensions of the odontoblasts. Maybe he will convince us someday on this point, may be he will convince himself otherwise."

DR. KITCHIN: "1. I think it would have been better to have made all your photographs at 4500× since this would have allowed a more accurate comparison between 'known' and 'unknown' structures since Neumann's sheath, if it exists would be larger than the enclosed dentinal fiber.

"2. The term *'fibril'* should not be used to designate the dentinal *'fiber.'* Fibril could well be reserved to designate the very fine fibers which form the dentin matrix.[4]

"3. A comparison of the unknown structures shown in *figs. 9* and *10* with the known dentinal fibers shown in *11*, *12*, *13*, and *14* would suggest to me that the latter are more nearly the size of dentinal fibers than Neumann's sheaths.

4. Since dentinal fibers do remain after the loss of all matrix material, then any case in which Neumann's sheath are also left would show up as a tube surrounding a tube, in other words a double ring when viewed in end section.

"I am unable to understand how dentinal fibers could be left after incineration unless they had undergone calcification. From what I have seen of over-decalcified dentin in rat molars, I am inclined to believe that what is left is a tubular extension of the odontoblast and predentin."

DR. SCHOUR: "Dr. Bodecker's report deals with a very difficult problem of technical procedure. In the nature of the methods employed in this investigation, the possibilities of artifacts that simulate the appearance of Tomes fibers are considerable.

"One could logically expect that the treatment of incineration to 1100°C. would result in the loss of all organic material, and the subsequent decalcification with 25% nitric acid would dissolve all minerals so that hardly any structural element would survive and there would at best remain only some debris. The examination at high magnification of such remnants in a solution of 25% nitric acid sealed with petroleum jelly may reveal artifacts that simulate the appearance of tubular structures. It is therefore important that control experiments be conducted. For example, the experiment of Method 2, consisting of subjecting a section of dentin first to incineration at 1100° and then to decalcification with 25% nitric acid could be repeated on a ground section of bone.

"In the dental literature the term "dentinal fibril" has been employed to refer to the very fine collagenous fibrils which make up the ultimately fibrous character of the dentin matrix. To use "dentinal fibril" as synonymous with the odontoblastic processes, "the Tomes fiber" or "dentinal fiber" may therefore be confusing."

DR. SICHER: "Incineration and decalcification have been used for a long time to isolate and to demonstrate the 2 components of bone and dentin. Incineration destroys the organic substance, decalcification removes the mineral content.

"Consecutive incineration and acid action on dentin destroys both components of the tissue and there is not the shadow of a doubt that any structures seen after this procedure can not be dentinal structures but are some extraneous matter. There is little doubt as to the nature of the tubules found by Bodecker. They are in all probability fragments of mycelia and spores of one of the common molds. Such molds are invariably found in distilled water and weak acids after long standing in a laboratory."

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[4] Editor's note. The term *fiber* has been substituted for *fibril* throughout the paper as published as we accept the former term as correct.

## CLOSING DISCUSSION

DR. BODECKER: The author believed, as did the consultants, that the temperature used in the above experiments (1100°C.) resulted in the complete destruction of the organic constituents of the specimens. Subsequent tests were made by incinerating dentin at a lower temperature but much longer periods (860°C. for 2–24 hours). Upon subjecting these specimens to 25% nitric acid, only an amorphous debris remained. It is evident therefore, that the incineration used in the original experiment resulted only in the destruction of the more volatile easily destructible organic structures of the dentin. Incineration and over-decalcification is a unique and admittedly illogical technique. Nevertheless, it has shown that the dentinal fiber, long mistaken for Neumann's sheath, is a slightly mineralized, organic structure, highly resistant to both acid and heat. This technique therefore, throws a new light on the structure and chemical constituents of the dentin.

The opinions of the consultants concerning the presence of Neumann's sheath are divergent. Dr. Schour is frankly skeptical. Dr. Cheyne states: "From the evidence presented, I am quite in agreement with the author who concludes that the structures are probably the remains of the odontoblastic fibers." Dr. Kitchin observes: "I am inclined to believe that what is left is a tubular extension of the odontoblast. . . ." Dr. Sicker takes a strongly skeptical stand. Referring to the structures shown in the photomicrographs illustrating this article, he states: "They are in all probability fragments of mycelia an dspores of one of the common molds. Such molds are invariably found in distilled water and weak acids after long standing in a laboratory." This statement is untenable. Molds could not survive in 25% nitric acid; also the solution was prepared weeks before the experiments. Besides, it is evident that the structures seen in *fig. 5* are dentin and not spores or mycelia. Here a small fragment of dentin is noted breaking up into its component tubules, which are shown in *figs. 7* to *10* after further action of acid had destroyed the dentin matrix as isolated dentinal fibers including an occasional lateral branch (*figs. 9* and *11*).

The discussion of Dr. Churchill is vague and contradictory. His experiment with the punctured sheet of celloidin, mentioned in this article, indicates his skepticism of the presence of a Neumann's sheath. It was, in fact, his work which stimulated the present investigation. The following quotations from Meyer's Histology translated by Churchill, (4) confirms his original stand. On p. 65: "the alleged sheath of Neumann" is mentioned; p. 62: "Interference and defraction lines have as it were magically produced the typical picture of the so called sheath of Neumann." Immediately following is a sentence in parenthesis to which Churchill added his name, indicating it was an expression of his own opinion: "(Besides these artifacts, other phenomena may simulate a sheath around the dentinal tubuli H. R. Churchill)." Finally on p. 67: "In our opinion no sheath of Neumann exists, but, as it is still described in recent literature, we thought it necessary to dwell more thoroughly on these much disputed structures, or rather artifacts." These quotations make Churchill's original stand clear.

The value of consultant articles, long running in the *Journal of Dental Education*, here again demonstrates its usefulness by the profitable discussion of the term "fibers." Drs. Kitchin and Schour suggest that "fibers" be restricted to the process of the odontoblast. This suggestion is welcomed as clarifying the terminology of the dentin. However, in order to show how wide spread is the use of "fibril," the following authorities are quoted. Orban carried through this terminology[1] while the following textbooks are at variance: Noyes, Schour and Noyes (2) p. 135 head a paragraph: "The Dentinal Fibrils (Tomes' fibers)"; Meyer-Churchill (1) p. 49 says: "This extension of the odontoblast, which after its discoverer is also known as Tomes' fibril . . ."; Bodecker (12) p. 29: "dentinal fibrils"; Dunning-Davenport Dictionary (13) offers this definition: "dentinal fibrils, the living fibers filling the dentinal tubules, see fibril of Tomes."

[1] Kitchin and Schour were both contributors to Orban's text—Ed.

Further, the designations of the fibrous, collagenous dentin matrix also differ: "collagenous fibrils (1); "fibrilar matrix" (14) "Fibrillae or fibrils" (2); "delicate fibrillae" (12).

Dr. Churchill still criticises the author's concept of the tubular character of the odontoblastic process. Additional evidence on dental lymph has been published (15) based on the vital staining experiments which further substantiate this concept.

The decision of the consultants is not unanimous, but the majority leans to the belief that Neumann's sheath is illusory. The vote is: Pro, Drs. Cheyne, Churchill, Kitchin and the author; skeptical: Drs. Schour and Sicher.

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# CHEMICAL STUDIES IN PERIODONTAL DISEASE. IV. PUTREFACTION RATE AS INDEX OF PERIODONTAL DISEASE[1]

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When the putrefaction rate of saliva is determined, values are obtained which seem to be related to the periodontal condition of the mouth of the patient from whom the saliva was taken. It has appeared that such saliva analysis might constitute a test for susceptibility to periodontal disturbances and that the test might be useful clinically as the now common salivary tests for caries susceptibility are useful. Accordingly, the present experiment was set up to determine how well the results of the salivary analyses correspond with clinical and radiographic findings in a fairly large group of patients.

## METHOD

For this experiment, 2 groups of 100 patients each were selected in the clinic of Northwestern University Dental School. Group I consisted of patients whose periodontal condition was apparently normal, as judged both by direct examination and by radiographs. Group II consisted of patients whose periodontal condition was abnormal and probably pathological. Some of these showed well advanced pocket formation, some with and some without apparent suppuration. Others showed only beginning pocket formation or a few isolated pockets. The radiographs of all patients in Group II showed some apparent loss of bone from around the roots of the teeth, or a definite loss of density of the bone at the interdental crests. The mouths of some of the patients in Group II were superficially clean; others were dirty. Degrees of cleanliness likewise varied among the patients in Group I, though they tended to have cleaner mouths. Patients in Group I would have been included in Groups 1 and 2, and those of Group II would have been included in Groups 3 and 4 as those groups were defined in the previous work[4].

Saliva was collected from each patient before any treatment was given. Each sample was obtained between 9 and 10 a.m. The patient was instructed to brush the teeth and eat breakfast on the morning when the sample was to be obtained, but not to use a dentifrice or a mouthwash, not to use any sugar and not to eat any highly flavored foods or mints or to chew any gum. Each patient collected

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[4] M. Berg, D. Y. Burrill, and L. S. Fosdick, Chemical studies in periodontal disease. III. Putrefaction of salivary proteins. J. D. Res. 25, 231, 1946.

about 35 cc. of paraffin-stimulated saliva in a sterile flask. The specimen was immediately divided into two 15 cc. portions in vials, 100 mg. of casein were added to each, all air bubbles were eliminated as described in the previous paper (1), the vials sealed, and both immediately placed in an agitating device in an incubator at 37.5°C. One portion was incubated for 1 hour and the other for 3 hours, at the end of which times the portions were removed for analysis. Analyses were carried out to determine the amount of hydrolysis (formol titration), and the amounts of indole and sulfides. The odor concentration of each portion was determined immediately upon opening the vial after incubation.

The mean values of all analyses, the standard deviation, and the percentage of cases falling within the range of standard deviation were computed for each group. Critical ratios were computed in a comparison of the 2 groups.

To express all values for each group (and for each patient) in a single figure, a periodontal disease index was devised, based on the formula A + 2B, where A stands for the values obtained from 1 hour incubation and B stands for those from 3 hour incubation. The figures are calculated in mil equivalents for hydrolysis and micro equivalents for indole and sulfides per 100 cc. of saliva. This difference in units corresponds approximately to the difference in the relative number of carboxyl groups to tryptophane and sulfide groups.

Assume the following values obtained on an analysis of saliva:

| | | | |
|---|---|---|---|
| One hour incubation A values | Formol titration | 3.0 cc. | = 3.0 mil eq. |
| | Indole | 1.0 $\gamma$ | = 0.81 micro eq. |
| | Sulfides | 1.0 $\gamma$ | = 3.13 micro eq. |
| | | | 6.94 |
| Three hour incubation B values | Formol titration | 4.0 cc. | = 4.0 mil eq. |
| | Indole | 2.0 $\gamma$ | = 1.62 micro eq. |
| | Sulfides | 3.0 $\gamma$ | = 9.38 micro eq. |
| | | | 15.00 |

The sum A + 2B = 6.94 + 30 = 36.94

Since the A values represent the status of cleanliness of the mouth, while the B values represent the speed of putrefaction, factor B was applied twice to minimize the influence of a "dirty" mouth. The median between the index values for normal and the index values for pathological saliva was found to be 17. Thus all indices above 17 are considered pathological. The above procedure was carried out for each patient. The formula was found to be useful in that it satisfactorily expressed the results of all analyses in a single figure.

The values for odor concentration were not included in the periodontal disease index because it was felt that the subjective nature of the measurements of odor placed them in a different category than the rest of the values.

The clinical and radiographic examinations were done for each patient before the chemical analyses of the saliva were started for that patient. The persons doing the chemical analyses did not know the results of the clinical examination until after the chemical analyses for that patient were done.

## RESULTS

The results of the analyses for the 2 groups, normal and pathological, the odor concentration values, and the computed means, standard deviation, percentages, and critical ratios are shown in Table I.

During the course of the work it was noted that, in general, saliva from patients in the pathological group would show high putrefactive values after 3 hours of incubation, whether the mouth was superficially clean or dirty. Saliva from a superficially clean mouth would give low values after 1 hour incubation, while that from a dirty mouth would give values almost as high after 1 hour incubation as after 3. Low values after 1 hour incubation and high values after 3 seemed to

Table I. Statistical Analyses of the Results of 200 Patients

| | HYDROLYSIS (FORMOL TITRATION) | | INDOLE | | SULFIDES | | pO | | PERIODONTAL DISEASE INDEX |
|---|---|---|---|---|---|---|---|---|---|
| | Hours | | | | | | | | |
| | 1 | 3 | 1 | 3 | 1 | 3 | 1 | 3 | |
| **100 normal patients** | | | | | | | | | |
| Mean | 1.33 | 1.89 | 0.265 | 1.10 | 0.27 | 0.605 | 3.20 | 5.20 | 11.81 |
| $\sigma$ | 0.26 | 0.38 | 0.21 | 0.67 | 0.15 | 0.38 | 1.13 | 1.36 | 3.82 |
| Range | 0.50–1.93 | 0.98–2.86 | 0–1.00 | 0.08–3.17 | 0–0.93 | 0–1.93 | 1–9 | 2–9 | 4.47–23.07 |
| Per cent within range of standard deviation | 73 | 73 | 85 | 65 | 81 | 74 | 88 | 75 | 68 |
| **100 pathological patients** | | | | | | | | | |
| Mean | 1.96 | 3.07 | 1.12 | 3.94 | 0.97 | 2.12 | 4.96 | 7.32 | 31.51 |
| $\sigma$ | 0.46 | 0.80 | 0.80 | 2.24 | 1.02 | 1.27 | 1.62 | 1.17 | 13.30 |
| Range | 1.15–3.77 | 1.83–6.34 | 0.15–4.44 | 0.58–14.10 | 0.08–7.7 | 0.30–7.70 | 2–9 | 5–9 | 14.99–89.55 |
| Per cent within range of standard deviation | 77 | 76 | 81 | 73 | 91 | 77 | 69 | 78 | 74 |
| Critical ratio | 17.80 | 19.83 | 15.32 | 17.97 | 10.04 | 17.00 | 13.13 | 17.52 | 21.11 |

indicate a high susceptibility to periodontal disease, not related to surface accumulations of debris.

As might be expected, treatment such as scaling, astringent packs, and the like, tends to reduce the periodontal disease index. The index appears to return toward its former values shortly after treatment unless the periodontal pockets have been largely eliminated and the patient's cooperation secured in maintaining the mouth in the corrected condition. Not enough cases of this type have been studied as yet, however, to justify a definite statement. Sugar, penicillin lozenges, and sulfathiazole paste have been tried in a few cases and appear to reduce the periodontal disease index temporarily.

Table II shows the periodontal disease indices of 3 normal and 3 pathological cases over 2 years' time, and the results of treatment of 5 of the cases. Treatment of the first normal case consisted of thorough scaling of the teeth. The second case was treated with 4 penicillin lozenges each day (2500 units each) for 3 days. The first pathological case was treated by scaling and use of astringent packs to reduce pocket depth. The second and third pathological cases used sugar, 1 cube at a time, 5 times a day for 7 days. Each cube weighed 7 grams. In each instance the saliva was obtained for analysis on the morning of the day following the last treatment.

Five patients having periodontal pockets apparently caused by occlusal interferences have been studied. In no case was the index high. The values were 14.9, 13.0, 14.9, 18.1 and 20.6. These values are considerably lower than would ordinarily have been expected for these patients. After cleaning and astringent packs, 2 cases gave values below the mean for normals. No analysis could be done after treatment of the other cases.

Table II. Pyorrhea indices for 3 normal and 3 pathological cases over 2 years' time, showing results of treatment in 5 cases

| | INITIAL | AFTER 1 YR. | AFTER 2 YRS. | AFTER TREATMENT |
|---|---|---|---|---|
| Normal | 7.45 | 8.67 | 10.62 | 6.65 (scaling) |
| | 9.90 | 11.38 | 9.00 | 7.24 (penicillin) |
| | 9.10 | 10.00 | 11.80 | No treatment |
| Pathological | 21.40 | 21.77 | 26.10 | 16.60 (scaling and packs) |
| | 41.20 | 62.60 | 68.20 | 27.50 (sugar treatment) |
| | 31.90 | 33.10 | 46.50 | 10.40 (sugar treatment) |

A number of other conditions seem to have some influence on the putrefactive rate of saliva. Inflamed third molar opercula produce an increased periodontal disease index, which becomes progressively higher as the infection and inflammation progress, and then drops again as the disease subsides. A cold or infected sinus with postnasal discharge causes a slight rise in the index. Vincent's gingivitis in a mouth where there are already periodontal pockets causes a rise in the index, but in an otherwise normal mouth may have little effect on it.

## DISCUSSION

In the previous paper, 4 groups were set up on the basis of increasing amounts of periodontal disturbance. Such fine distinctions are difficult to make and confusion may exist between any one group and the next one. Between normal and pathological mouths, however, the distinction is clearer and the chance for confusion much less. The validity of the clinical and radiographic diagnosis and the value of the saliva analyses was unexpectedly confirmed by the occurrence of 3 instances in which a second clinical and radiographic examination, following a year or more after the first, seemed to show a marked improvement in the

general mouth condition. Interdental bone appeared in the second radiograph where the first seemed to show marked resorption. It was thought that there had been an error in diagnosis, but the saliva analyses entirely confirmed the clinical and radiographic findings. In each case the periodontal index had dropped markedly. This correlation was discovered only after all results were at hand. Correspondingly, cases were found where saliva and x-rays showed increased signs of periodontal disease.

Probably the differences in the purefactive rates of the saliva of normal and pathological cases arise from differences in the bacteria present when periodontal detachment occurred. No attempt is made on the basis of the present material to make any definite statement about etiology. It seems clear, however, that periodontal detachment and formation of pockets is associated in almost all cases with an increase in the rate of putrefaction of the saliva. If further studies confirm these results, it may be possible to predict whether a patient is susceptible to periodontal disease and in need of preventive therapy, just as it is now possible to predict with fair accuracy whether a patient is susceptible to dental caries and requires preventive measures.

## SUMMARY AND CONCLUSIONS

Analyses have been made of the putrefactive rates of the saliva of 100 patients with normal periodontal conditions and 100 patients with abnormal or pathological periodontal conditions.

It was found that saliva from pathological cases putrefies more rapidly than saliva from normal cases. The correspondence is high between radiographic and clinical findings on the one hand and chemical findings on the other.

A periodontal disease index has been devised and its validity demonstrated.

# DEVELOPMENT OF RAT MOLAR CROWNS AND JAWS[1]

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Brash (1) used the madder feeding technic (in the classical manner of John Hunter) to study the growth of the pig's skull. This vital staining method provided direct evidence of the sites of bone growth. He showed that the change in the occlusal plane is not a mere alteration of level from a fixed plane at the base of the skull, but a continuous alteration from both maxillary and mandibular sides by constant additions to actual surfaces of alveolar borders.

Hoffman and Schour (2) have made a contribution to the problem of tooth and bone growth using a refined quantitative method of alizarin staining which "demarcates fixed bony points or levels from which subsequent relative positions of the tooth may be measured." Their staining method obviously has advantages over Brash's. Their study fully described rat molar development and eruption by gross and radiographic analysis (from 13 to 1050 days) and by vital staining with alizarin Red S (from birth to 500 days). They stated that "eruption is continuous throughout the period studied. It begins with the first evidence of calcification. The path of eruption is in an occlusal, anterior, and lateral direction. Fastest rates are found prior to 35 days of age. The rate of eruption equals the sum of the rates of root elongation and of fundic bone apposition and decelerates with age. . . . If there were any downward *migration* we would find resorption of fundic alveolar bone instead of apposition."

But in histologic examination of the fundus of the crypts enveloping the developing crowns of rat molars in the first week of life, we do find resorption of bone (*fig. 3*). This resorption however, indicates downward growth and not downward migration. This problem revolves around the understanding of the epithelial sheath which Orban (3) described as a relatively fixed point in the development of the teeth. The original error was due to the floor of the nose having been regarded as stable and additionally to a misconception of the nature of the epithelial sheath. In a previous study (4) it was demonstrated that the region designated as the cervical loop was concerned with forming the future cervical region of the crown. The epithelial sheath is not in evidence until the crown of the tooth is virtually completed. The epithelial sheath does not begin to function until a later stage of development. Only when the cementoenamel junction is reached is the epithelial sheath a relatively fixed point as the tooth erupts in the direction of the oral cavity. Until the functional stage of the epithelial sheath begins, the crown of the tooth develops in a fixed site

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increasing in size eccentrically in all directions including the fundic area. The evidence of resorption of bone in the fundic area supports this view.

Hoffman and Schour (2) published an x-ray study of rats from 13 to 1050 days but left out the early stage of crown development under discussion here. Spence (5) published an x-ray study of the rat beginning at birth. His method has the advantage of using the same animal, but the disadvantage of lack of detail in the early stages due to the hard x-rays used. Because of their high penetrability, such rays are capable of differentiating only marked variations in radiopacity.

## FINDINGS

The difficulty of demonstrating detailed changes with ordinary hard x-ray apparatus is seen in *fig. 1*, where a series of dissected mandibles 1 day, 15 days, 30 days, and 200 days old were arranged and x-rayed simultaneously. The mandible in the first day of life is indistinct in the slightly enlarged x-ray nega-

*Fig. 1.* X-ray study of a series of dissected mandibles, 1 day, 15 days, 30 days and 200 days old. Note that mandible in first day of life is indistinct using ordinary clinical x-ray apparatus. Density (clarity) varies directly with increase in age.

tive, but the later stages are clearer in direct proportion to their density. By the use of an x-ray tube especially constructed to operate at potentials in the neighborhood of 10,000 volts, (6) it is possible to see details of the early stages of crown formation which cannot be seen otherwise.

*Fig. 2* shows soft x-ray pictures of dissected mandibles of 1, 2, and 3 day old rats from the same litter. We now can see that 3 groups of bone fibers radiate from the symphysis, one toward the angle, one into the condyle, and another into the coronoid process. There also seems to be a separate ossification center or mechanism for the molars and incisor. The pictures (all same enlargement) demonstrate an overall increase in the size of the rat mandible each day. There is also a progressive enlargement of each ossification center and of the tooth crowns and their crypts.

When these soft x-ray negatives are compared with decalcified sections of rats of the same age, we see that there is an adaptive bone resorption in the fundus of the crypts, as well as progressive enlargement of the mandible. That is to say, there is resorption of fundic bone *synchronized* with enlargement of

the tooth crown, as well as an overall increase in the size of the mandible by constant additions to its outer surfaces. *Fig. 3* is a low power photomicrograph

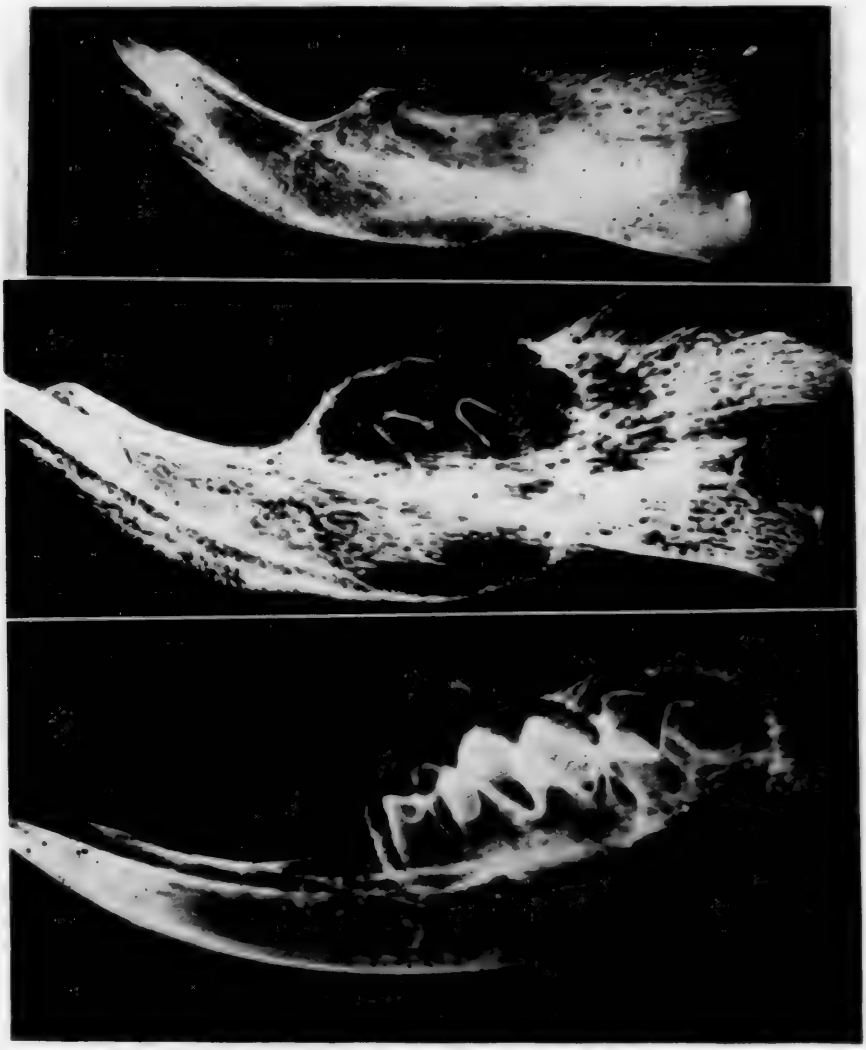

*Fig. 2.* Soft x-ray study of series of dissected mandibles of 1, 2, and 3 day old rats. Note that detail can be seen in these young mandibles. Negatives show overall increase in size of rat mandibles each day and progressive enlargement of tooth crowns and their crypts.

of a sagittal decalcified section of a mandibular molar crown of a 2 day old rat. The enamel organ is surrounded by the tooth follicle and bone.

Under higher magnification of the section in *fig. 3* show the cervical loop

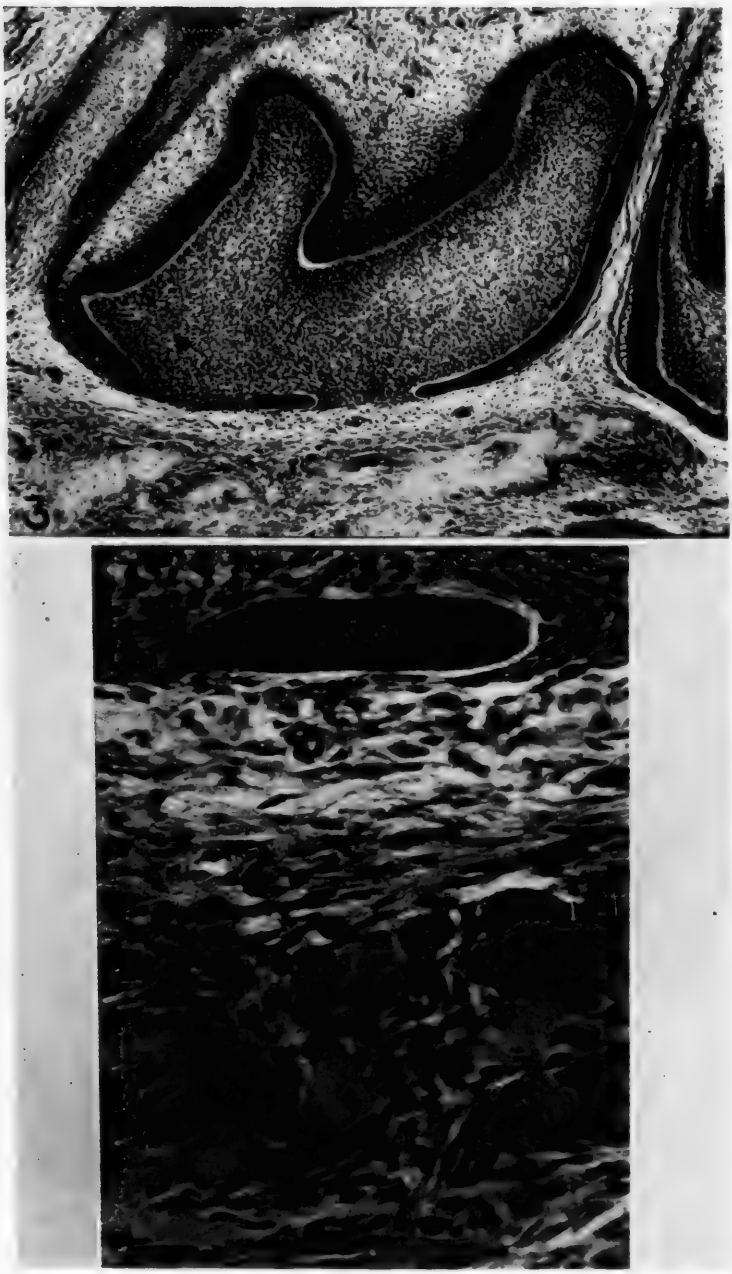

*Fig. 3.* Low-power photomicrograph of sagittal decalcified section of mandibular molar of 2 day old rat. Note enamel organ, tooth follicle and bony crypt.

*Fig. 4.* High power photomicrograph of loop of epithelium (future bifurcation) of enamel organ seen in *fig. 3*. Resorption of fundic bone is evidenced by osteoclastic action correlated with enlargement of tooth crown.

region of the enamel organ of a molar tooth can be seen (*fig. 4*). Resorption of bone at the fundus of the tooth crypt is evidenced by osteoclastic action which is correlated with the enlargement of the tooth crown.

## DISCUSSION

It appears that one must correlate different methods of study in order to gain an understanding of growth of rat molar crowns.

It has been demonstrated in many ways that there is a remodeling of the jaw bones by deposition and resorption of bone during growth. It has also been demonstrated that eruption of the developing teeth is due largely to bone deposition at the fundus of the alveolus plus the growth in length of the roots.

But it is now maintained that this eruptive process only begins after the completion of the crown of the tooth and the start of root formation. While it was assumed that the process of fundic bone deposition occurred even in the earliest stages of tooth formation, study of the fundus of the developing tooth crown shows bone resorption. This resorption continues until the full size of the crown is attained. Only with the beginning of root formation is the epithelial sheath a relatively fixed point; bone resorption ceases, apposition commences, and the tooth begins to move out of its socket. This mechanism is similar to that of the human (8).

A natural experimental check on these findings occurs in the remarkable mutation called the grey lethal mouse (7). These mice have profound anomalies in skeleton and teeth just because they lack the power of bone resorption. As a consequence their teeth never erupt; in fact, the developing crown is compressed by the unyielding bony crypt.

As has been pointed out, the size and shape of the tooth crypt is determined by the growth of the tooth crown in normal mammals. But in the grey lethal mouse, due to this hereditary lack of the power of bone resorption, the size and shape of the tooth crown is limited by that of the crypt at the time the tooth becomes enclosed in it. Teeth, which still have to grow after that time, are actually deformed due to the inability of the bone to resorb in response to the stimulus of the growing crown.

The lower incisors of these mice, instead of growing in the normal way—backwards, till they reach the base of the condyloid process—find a way out of the mandible through the mental foramen. . . Here again the bone does not yield to the stimulus of the backward growing end of the tooth. This situation is exactly complementary to the normal course of events.

## SUMMARY

Evidence has been presented to indicate that there is no fixed point in development of the teeth and jaws of the rat. In early stages of crown formation there is actually bone resorption with expansion of tooth crown and follicle deeper into the jawbone prior to eruption. After completion of the crown there is movement of the tooth outward. The mechanism is similar to the human.

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# CHEMICAL CHANGES IN THE DENTIN FOLLOWING AGEING AND PERIPHERAL IRRITATION[1]

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In the present study, 2 types of primary dentin were analyzed. Histological examination of ground sections of human teeth has shown that there are comparatively opaque areas in primary dentin which have been variously termed metamorphosed dentin (6), sclerotic dentin (3), dead tracts (8), and attrition cones. These opaque areas are thought to represent a change in the primary dentin in response to age and/or exposure. Attempts have been made to demonstrate the increased density of these areas by the use of the x-ray (9, 10), and the Grenz-ray (2). The results generally seem to indicate an increased density. In the present study an attempt was made to determine if a difference in chemical composition between the opaque and the "normal" dentin existed. For simplicity, the "normal" dentin will be referred to as Type I, and the "opaque" areas will be referred to as Type II. If Type II is comparatively caries resistant as claimed (1, 5), some light may be shed on the mechanism of caries immunity.

## EXPERIMENTAL PROCEDURE

### *1. Selection of the sample*

Teeth selected at random were used. With a few exceptions, they were extracted for reasons unknown to the authors. They were fixed in formalin for an indefinite period, never less than 1 week. Ground sections less than 0.5 mm. thick were prepared using the apparatus described by Bodecker (4). The enamel, cementum, pulp remnants, and predentin were then removed with a #556 crosscut fissure bur. The sections were examined microscopically and Type I and Type II areas were separated with the aid of a #556 crosscut fissure bur. The samples of each type obtained from each tooth were pooled so that the analytical sample represented all of the dentin of one or the other type obtainable from a single tooth.

### *2. Chemical*

Each sample was heated at 100°C. for 48 hours and was then transferred to a desiccator. They were weighed in porcelain crucibles which had been previously brought to constant weight by heating, and were then ashed. Ashing was done

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TABLE I

*Percentage of ash, calcium, and phosphorus in dentin*

| SAMPLE | | ASH % OF DRY WEIGHT | | CALCIUM % OF ASH WEIGHT | | PHOSPHORUS % OF ASH WEIGHT | |
|---|---|---|---|---|---|---|---|
| No. | Type | Value | Diff. | Value | Diff. | Value | Diff. |
| 2 a | 1 | 65.91 | | | | | |
| | 2 | 67.79 | 1.86 | | | | |
| 3 b | 1 | 61.13 | | 42.70 | 4.91 | 16.01 | 0.90 |
| | 2 | 66.67 | 5.54 | 37.79 | | 15.11 | |
| 4 b | 1 | 72.5 | | 41.18 | 0.09 | 18.4 | 0.35 |
| | 2 | 73.8 | 1.3 | 41.09 | | 18.05 | |
| 5 a | 1 | 60.58 | | 41.37 | 2.96 | 16.78 | 2.34 |
| | 2 | 64.79 | 4.21 | 38.41 | | 14.44 | |
| 6 a | 1 | 72.90 | | 31.99 | 0.19 | 16.63 | 0.99 |
| | 2 | 76.46 | 3.56 | 31.80 | | 15.64 | |
| 7 b | 1 | 72.37 | 0.45 | 38.13 | 0.00 | 16.37 | 0.48 |
| | 2 | 71.92 | | 38.13 | | 15.69 | |
| 8 b | 1 | 70.15 | | 44.02 | 5.03 | 16.65 | 0.23 |
| | 2 | 71.88 | 1.73 | 38.99 | | 16.42 | |
| 10 b | 1 | 70.69 | | 34.02 | 2.12 | 19.7 | |
| | 2 | 74.23 | 3.56 | 31.90 | | 19.9 | 0.2 |
| 12 b | 1 | 70.87 | | 42.74 | 5.84 | 18.71 | |
| | 2 | 71.15 | 0.28 | 36.90 | | 18.80 | 0.09 |
| 13 a | 1 | 70.59 | | 37.09 | 0.71 | 17.40 | |
| | 2 | 73.52 | 2.93 | 36.38 | | 17.68 | 0.28 |
| 14 a | 1 | 70.51 | | 45.59 | 3.58 | 17.9 | 1.60 |
| | 2 | 72.48 | 1.97 | 42.01 | | 16.3 | |
| 15 b | 1 | 69.00 | | 34.22 | 6.20 | 19.02 | 4.16 |
| | 2 | 72.67 | 3.67 | 28.02 | | 14.86 | |
| 16 a* | 1 | 69.76 | | 39.43 | 6.84 | 28.9 | 18.5 |
| | 2 | 81.73 | 11.95 | 32.59 | | 10.6 | |
| 17 | 1 | 69.46 | | 41.60 | 0.50 | 18.30 | |
| | 2 | 73.03 | 3.57 | 41.12 | | 18.75 | 0.45 |
| 18 b | 1 | 70.40 | | 38.80 | 0.21 | 18.35 | 0.15 |
| | 2 | 76.50 | 6.10 | 38.59 | | 18.20 | |
| 19 a | 1 | 71.80 | | 42.75 | 2.01 | 18.5 | |
| | 2 | 74.60 | 2.80 | 40.74 | 18.7 | 18.7 | 0.20 |

TABLE I—*Continued*

| SAMPLE | | ASH % OF DRY WEIGHT | | CALCIUM % OF ASH WEIGHT | | PHOSPHORUS % OF ASH WEIGHT | |
|---|---|---|---|---|---|---|---|
| No. | Type | Value | Diff. | Value | Diff. | Value | Diff. |
| 21 b | 1 | 68.41 | | 39.60 | 0.00 | 18.66 | 0.55 |
| | 2 | 70.20 | 1.79 | 39.60 | | 18.11 | |
| 23 a | 1 | 65.83 | | 38.99 | 0.48 | 17.78 | 0.50 |
| | 2 | 70.51 | 4.68 | 38.51 | | 17.88 | |
| 24 b | 1 | 66.41 | | 39.9 | | 17.94 | 0.22 |
| | 2 | 70.32 | 3.91 | 40.15 | 0.25 | 17.72 | |

a—Carious
b—Caries-free
*—Deciduous

by 2 methods. Some samples were incinerated over a bunsen flame with concentrated nitric acid. Others were ashed by placing them in an electric oven for 96 to 120 hours at 850°C. to 900°C. Some samples were crushed before ashing. Pairs of samples from each tooth were treated similarly. They were then weighed to determine the ash weight. The residues were dissolved in hydrochloric acid, transferred to 50 ml. volumetric flasks, and diluted to volume with water.

The calcium and phosphorus determinations were done by the methods previously described by Dragiff and Karshan (7). Each determination was done in duplicate. The results quoted in the table are averages of the 2 results.

## RESULTS

The analytical results given in Table I clearly indicate that there are significant differences between the 2 types of dentin. Type II differs from Type I in that there is, in all but 1 of the teeth analyzed, a greater percentage of inorganic matter in Type II; in the inorganic matter, in all but 3 teeth there is a lesser percentage of calcium, and in all but 5 cases a lesser percentage of phosphorus, in Type II dentin.

The dentin of Type II associated with caries is similar, in composition and in its deviation from Type I, with the dentin of Type II associated with age and exposure.

The possible errors are of 2 types. The first ivolves the sample itself, and the second involves the analytical procedures.

The teeth were formalin fixed after extraction. It is possible that this may have affected the organic matter of the dentin. However, inasmuch as each tooth is its own control, this source of error is neutralized. There is also an error in the selection of the sample. The change of dentin from Type I to Type II is never complete in any area (3). The opacity of Type II dentin will, under low magnification, mask any Type I dentin which may be present in the area. Type I

dentin will never mask Type II. Therefore, if this error does exist, it would imply that the actual change is greater than that which has been noted.

There are various errors involved in the analytical procedures. The error involved in the ashing process is a small one and is only a weighing error. It is approximately 0.1%. The average error of the calcium determination as calculated from the average precision of the duplicates is about 1.5%. The average error in the phosphorus determination, calculated in a similar fashion, is about 1.5%.

## DISCUSSION OF RESULTS

From the analytical results it appears that some inorganic substance which is not calcium or phosphorus is being incorporated into the dentin to produce the change which has been noted above. The nature of the substance which is being added is not indicated. Further work is in progress, however, to ascertain its nature.

In the case of the one deciduous tooth analyzed, the ash, calcium, and phosphorus percentage changes are in the same direction as, but much greater than, the corresponding changes in the permanent teeth.

## SUMMARY AND CONCLUSIONS

1. Analysis of 1 deciduous and 18 permanent teeth indicates that definite differences in chemical composition exist between the 2 types of dentin analyzed. Type I is the "normal" dentin which is translucent to direct light in ground section. Type II, also called metamorphosed or sclerotic, is opaque in direct light in ground section. There is a lesser percentage of organic matter, and a decreased percentage of calcium and of phosphorus in the ash, in Type II dentin, as compared with Type I.
2. The change appears to be one of deposition of an undetermined substance.
3. Work is in progress to determine the nature of the materials deposited.

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# A COMPARATIVE STUDY OF THE EFFECTS OF GLYCERITE OF HYDROGEN PEROXIDE AND OF HEXYLRESORCINOL ON THE BACTERIA OF THE NORMAL MOUTH[1]

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## INTRODUCTION

Over fifty years ago, Miller (17) explored the possibility of using antiseptic solutions to control the number of oral organisms, reporting that none of the agents then available satisfactorily accomplished this purpose. More recently, Feirer and Leonard (8) tested twenty-seven commercial mouth preparations *in vitro* against *Staphylococcus aureus*, and reported that none was bactericidal although in contact with the organism for more than five minutes. Mead (15) has published similar results.

Of the many "antiseptic mouth washes" used, very few possess true bactericidal properties. Among those which are effective must be listed S.T. 37, (8), Metaphen (5), Solution 58 (11), sodium ricinoleate (15), sodium alkyl sulfate (15), the cationic detergents (18), and the peroxides. These last, concerning which there has been much controversy, are frequently the subject of enthusastic report. On the basis of clinical studies, hydrogen peroxide has been recommended by Farrell (7), and zinc peroxide by Meleney (16) and by Mallett and Guralnick (14). Although more stable than aqueous hydrogen peroxide in the presence of organic matter, zinc peroxide requires heating to 140°C. for 4 hours before using and leaves a gray coating lining the oral tissues. Hydrogen peroxide in aqueous solutions is relatively unstable and is so quickly decomposed by peroxidase, catalase and other enzyme systems, that its antiseptic effects are negligible. It is, however, detergent, deodorant and hemostatic.

In order to counteract the instability of aqueous peroxide solutions it occurred to Brown, Abramson, Gorin, Kauffman and Shanley (2) that urea peroxide could be used as a source of hydrogen peroxide if dissolved in a water-free vehicle. With the discovery that urea peroxide was both soluble in anhydrous glycerol but also more stable when so dissolved, (2) it appeared that peroxides in such solutions might possess unusual antiseptic properties.

Since trace elements may be present in glycerol and act as catalysts affecting peroxides, a secondary stabilizing agent, 8-hydroxyquinoline (0.1%), must be

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added. This substance reacts with metals to make non-ionizeable salts which not only protect the peroxide from the metallic impurities occasionally present in glycerol, but also renders metallic elements incapable of being used by microorganisms for their normal metabolism (24). The solution used consists, therefore of hydrogen peroxide (1.5%) as derived from urea peroxide (4%) in substantially anhydrous glycerol with 8-hydroxyquinoline (0.1%).

Glycerite of hydrogen peroxide has the physical characteristics of glycerol. Its surface tension at 20°C. is 65.6 dynes/cm. (water 72.25 dynes/cm.). It is slightly yellow in color. Its approximate pH is 6.0. It is miscible with water in all proportions. Its stability has been the subject of a separate study, (2) in which it was shown by titration methods that samples left in clear bottles in and out of sunlight were stable for periods beyond 1 year. The properties of the individual ingredients merit brief mention.

Glycerol would seem to be an ideal vehicle for an oral preparation. It has a pleasant taste and no ill effects follow its ingestion (12) since it can be taken orally by humans in doses of 30 cc. 3 times daily for as long as 50 days. It is miscible with saliva of which it increases the flow. It seems, however, to line the oral mucosa with a film which serves to bring the antiseptic into close contact with the tissues. Its viscosity limits the rate of decomposition of the peroxide which as it decomposes renews the interface between the solution and the tissues prolonging the antiseptic action. As reported by Rodriquez (20) glycerol has a protective action against the irritation of antiseptic substances, such as iodine.

Since urea peroxide is decomposed by water into urea and hydrogen peroxide, each substance requires separate discussion.

Urea, of which 20 grams can be ingested by human beings up to 5 times daily (9) plays a special part in oral preparations. Normally a constituent of saliva, the urease present in the mouth rapidly converts it into ammonium carbonate which acts to buffer any acids which may be present. When Grove and Grove (10) noted that the dental caries was retarded when salivary ammonia was high, it was logical to suggest the use of ammonia locally to diminish plaque formation. Stephan (22) then suggested the direct use of urea for this purpose. Further experiments by Wach, O'Donnell and Hine (23) indicated that urea (4%) was an efficient buffering agent for acids present in the mouth. It should be noted in this regard that urea is a peptizing agent for damaged tissue and actually promotes healing (19) in suppurative conditions.

Hydrogen peroxide has a long history in both the art and science of dentistry. Since it is non-toxic and relatively non-selective in its action upon bacteria (3) affecting Gram positive and Gram negative organisms and since as an oxidizing agent it possesses excellent deodorizing properties, it would seem to be an ideal substance for oral antisepsis. Although the mechanical effects of its foaming action are considered desirable for dental work, its relative instability in storage and its transient action in the presence of many catalysts mitigates against its use as an antiseptic. In glycerol the hydrogen peroxide derived from urea peroxide is more stable in vitro and decomposes less quickly in vivo and when in contact with oral tissues. Its use as a dentrifice and as a mouth wash (diluted with water 50%) for periods up to 23 months on 83 individuals both with normal

mouths and with oral infections, has shown no cases of irritation or of "hairy tongue".

Studies concerned with the action of glycerite of hydrogen peroxide of representative groups of bacteria, comparing it with 7 commonly used antiseptic solutions, showed it to be an effective bactericide by laboratory methods (3). The solution was also found to be highly efficient as well as non-irritating and non-allergenic in a series of 800 patients upon whom it was used as a topical application for a wide variety of infections of the skin, mucous membranes and specialized epithelia (1). In a previous communication it was shown to have a marked germicidal effect upon the type and numbers of oral microorganisms (21).

Workers in this field must be fully cognizant of the fact that there is no completely satisfactory method of evaluating the efficacy of an antiseptic solution used in the mouth. The oral cavity is an ideal incubator and varies in its flora from hour to hour and from day to day. The variation in numbers and types of the organisms present depend upon the diet, the number of meals and their composition as well as the flow of saliva and its composition. There is as well as "diurnal tide" as noted by Feirer and Leonard (8) and explained by Crowley and Rickert (6) as due to a resurgence following the reduction caused by the mechanics of mastication. Although subsampling methods are not to be relied upon completely in testing mouth washes, yet the effects of antiseptic solutions upon oral organisms can only be tested by using them in the mouth, and subjecting the experimental and control data to statistical study.

The number of bacteria present before and after the use of an antiseptic solution may bear no direct relationship to the total number present but a marked change in the type of bacteria or a noteworthy decrease in their numbers suggests that the antiseptic is having some effects especially when these are consistent for each solution studied and compared with controls.

## EXPERIMENTAL METHODS

For the purpose of the present study, the following technique was used. Immediately after lunch the subject expectorates into a sterile test tube. One milliliter of the saliva is accurately measured into 99 ml. of sterile tap water in a screw-cap bottle using a graduated pipette designed to deliver 1.0 ml. After introducing the saliva into the dilution bottle the pipette is rinsed once in the water and the rinsings returned to the bottle. This serves to ensure complete delivery of the measured amount of saliva and is necessary since with samples of high mucin content the delivery is otherwise incomplete. The diluted sample is now shaken 25 times through an arc of 1 foot in 7 seconds, thereby securing a uniform, essentially reproducible distribution of the organisms present. One milliliter of this dilution is added to a second bottle containing 99 ml. of sterile tap water, using a sterile pipette. This second ilution is shaken as above and with a third sterils pipette, samples (1.0 ml. and 0.1 ml.) are plated from it into 15 ml. of nutrient agar, care being taken that the tip of the pipette is free from drops. This is particularly important to insure accurate measurement of the smaller amount.

The subject is next given 25 ml. of either of the antiseptic solutions described

below, holding each material in his mouth (with occasional swishing between the teeth) for a period of 5 minutes, at the end of which time the antiseptic is discharged. Ten minutes later and again at the intervals of 1, 2 and 3 hours, the subject expectorates into sterile test tubes and platings of the saliva are made as described above.

The plates are incubated for 48 hours at 37°C., counted, and the bacterial count per milliliter of the original saliva calculated from the dilution used. The dilutions suggested (1:10,000 and 1:100,000) have generally yielded plates suitable for counting. Control tests are made on separate days, the antiseptic solution being replaced in the procedure by 25 ml. of sterile diluted water.

It should be noted that no artificial stimulation of the flow of saliva, as by chewing paraffin, is permitted since it has been shown by Knighton (13) and other workers that the chewing of paraffin in itself exerts a cleansing action. For the same reason, the use of a tooth brush was not introduced into any examination of a preparation being studied for its effects as a mouth wash, especially since brushing of the teeth does not ordinarily accompany the use of an oral antiseptic by the dental surgeon.

The method does not demonstrate the action of an antiseptic solution upon any particular groups of bacteria such as the acidogenic microorganisms, the spirochetes, or the fusiform bacilli but rather illustrates only the effects upon those organisms ordinarily capable of growing in nutrient agar, although these are not necessarily pathogenic. Separate studies (4, 21) have shown the solution to be effective both by cultural and clinical methods for the oral pathogenic organisms.

In view of the extensive literature concerned with hexylresorcinol (S.T. 37) and its effect upon oral bacteria, it was chosen for comparison with glycerite of hydrogen peroxide. Both solutions were diluted with an equal amount of water immediately before use, respectively representing hexylresorcinol 1:2,000 and hydrogen peroxide 1:33 (0.75%) in glycerol (50%).

The hexylresorcinol preparation in the strength in which it was employed was decidedly irritating to all of the individuals studied. The "anesthesia" of the tip of the tongue, mentioned by Feirer and Leonard (8) was noted. The sense of taste was affected in all of the subjects for periods of 2 to 7 days. This excessive irritation was undoubtedly due in part to the unusually long period of contact (5 minutes) with the oral tissues.

On the other hand, the glycerite of hydrogen peroxide solution was used without complaint in all but 2 of the subjects, the only sensation which occurred being one of "tingling". This was related to the release of oxygen and ceased immediately upon discharge of the antiseptic solution from the mouth. Two subjects noted symptoms of transient pain while the antiseptic was in contact with exposed dentine. This was shown to be due to the glycerol content and neither subject was affected when the glycerol was reduced from 50% to 33%.

## EXPERIMENTAL DATA

It will be noted from Table I that in every 1 of 10 experiments using the glycerite of hydrogen peroxide, the bacterial count showed a marked diminution in

10 minutes (average 14.3% of the original level) following which the bacteria show a steady increase in numbers 1, 2 and 3 hours afterwards. In 4 experiments the 3-hour count approximates or is greater than the initial count. The average

TABLE I

*Percentage of original number of organisms remaining in the mouth following a five minute rinse with hydrogen peroxide (0.75%) in glycerol (50%)*

| | EXPER. NO. | | | | | | | | | | AVERAGE OF 10 TESTS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| | | | | | | | | | | | % |
| Preliminary.... | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 10 minutes..... | 9.4 | 17.6 | 5.7 | 2.5 | 11.3 | 21.6 | 31.0 | 30.7 | 13.7 | 7.4 | 14.3 |
| 1 hour..... | 24.4 | 31.2 | 6.6 | 11.9 | 68.4 | 37.2 | 34.1 | 36.0 | 28.4 | 16.8 | 25.1 |
| 2 hours..... | 40.9 | 28.0 | 21.4 | 40.7 | 129.7 | 62.7 | 50.0 | 72.0 | 116.6 | 19.3 | 40.1 |
| 3 hours..... | 203.1 | 31.4 | 83.4 | 89.8 | 124.1 | 94.1 | 51.0 | 120.0 | 104.8 | 76.9 | 72.1 |

TABLE II

*Percentage of original number of organisms remaining in the mouth following a five minute rinse with S.T. 37 (1:2000)*

| | EXPER. NO. | | | | | | | | | | AVERAGE OF 10 TESTS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| | | | | | | | | | | | % |
| Preliminary.... | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 10 minutes..... | 5.9 | 28.1 | 95.5 | 15.4 | 26.7 | 21.9 | 35.6 | 62.0 | 22.9 | 37.6 | 29.8 |
| 1 hour..... | 36.6 | 81.7 | 273.5 | 64.1 | 224.6 | 22.6 | 117.6 | 68.5 | 50.4 | 153.4 | 93.3 |
| 2 hours..... | 37.6 | 97.4 | 287.0 | 111.4 | 229.5 | 83.8 | 115.1 | 159.2 | 91.0 | 116.7 | 107.0 |
| 3 hours..... | 84.5 | 164.8 | 352.8 | 110.2 | 244.0 | 133.0 | 140.6 | 163.9 | 113.0 | 146.6 | 143.4 |

TABLE III

*Percentage of original number of organisms remaining in the mouth following a 5 minute rinse with distilled water*

| | EXPER. NO. | | | | | | | | | | AVERAGE OF 10 TESTS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| | | | | | | | | | | | % |
| Preliminary.... | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 10 minutes..... | 136.5 | 163.0 | 77.5 | 79.6 | 198.7 | 113.3 | 104.3 | 507.5 | 102.9 | 153.3 | 124.4 |
| 1 hour..... | 620.0 | 128.3 | 88.9 | 97.5 | 164.3 | 381.0 | 140.1 | 431.0 | 113.9 | 192.2 | 157.8 |
| 2 hours..... | 610.0 | 150.5 | 99.8 | 189.8 | 233.9 | 649.0 | 167.3 | 538.0 | 123.6 | 150.7 | 176.0 |
| 3 hours..... | 352.0 | 354.0 | 123.1 | 231.3 | 362.7 | 933.0 | 113.0 | 523.0 | 114.4 | 198.7 | 336.7 |

of all of the estimations, however, is 72.1% with the initial count taken as 100%. For a subsampling technique, open to great variation, the results are remarkably consistent, confirming the earlier communication concerned with this solution (21).

In Table II are listed the results following the use of S.T. 37. The 10-minute count shows a decrease which averages 30% of the original level. Within an hour the bacteria have reached or surpassed the initial level in 4 experiments. By the second and third hours, the number of bacteria is greater than the number present before using the solution, the average reaching 143%.

Table III illustrates the results following control experiments with a water rinse which has little effect upon the number of bacteria present, only two of the counts demonstrating a slight decrease in the numbers of bacteria normally found in the "diurnal tide", the three hour count reaching an average of 336%.

Table IV demonstrates the control data when neither a meal nor water nor a

TABLE IV

*Normal per cent increase in the number of organisms present in the mouth during a test period (no food, water or medication)*

| | EXPER. NO. | | | | | | | | | | AVERAGE OF 10 TESTS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| | | | | | | | | | | | % |
| Initial count... | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| 1 hour..... | 233.9 | 287.6 | 36.1 | 234.1 | 366.7 | 339.6 | 191.0 | 147.2 | 205.5 | 216.9 | 233.9 |
| 2 hours..... | 337.3 | 216.9 | 429.2 | 642.7 | 509.5 | 1340.3 | 336.9 | 234.7 | 393.5 | 341.3 | 371.4 |
| 3 hours..... | 419.6 | 405.3 | 491.3 | 793.9 | 804.0 | 1053.4 | 377.1 | 393.5 | 325.5 | 406.0 | 445.5 |

TABLE V

*Percentage variation in the number of organisms present in the mouth as a result of the ingestion of food (the diurnal tide)*

| | EXPER. NO. | | | | | | | | | | AVERAGE OF 10 TESTS |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| | | | | | | | | | | | % |
| Before lunch... | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 | 100.0 |
| After lunch.... | 37.8 | 22.5 | 28.8 | 8.2 | 12.6 | 14.4 | 27.9 | 24.8 | 20.0 | 36.6 | 23.9 |
| 1 hour..... | 88.4 | 64.7 | 68.0 | 19.2 | 46.2 | 48.9 | 53.3 | 36.5 | 41.1 | 79.4 | 56.0 |
| 2 hours..... | 127.5 | 48.8 | 123.6 | 52.7 | 64.2 | 193.0 | 94.0 | 58.2 | 78.7 | 124.9 | 88.9 |
| 3 hours..... | 158.6 | 91.2 | 141.5 | 65.1 | 101.3 | 151.7 | 105.2 | 97.6 | 65.1 | 148.6 | 106.6 |

medicated mouth rinse is used. It will be observed that with the initial count taken as representing 100% there are successive hourly percentage increases which average 233.9%, 371.4% and 445.5%.

Table V shows the effects of a meal upon the numbers of oral bacteria. The initial count is diminished to an average level of 23.9% with respective hourly increases of 56.0%, 88.9% and 106.6%, at the end of 3 hours. These data corroborate the work of Crowley and Rickert (6) and serve also as a type of control experiment.

For the purpose of the present study, the percentage data of Tables I and II should be compared with those listed in Table IV, from which it may be con-

cluded that the effects of both antiseptic solutions are greater than would appear from a literal interpretation of the data presented.

*Fig. 1* illustrates more graphically the effects of a water rinse, the use of S.T. 37, and of glycerite of hydrogen peroxide; the broken line demonstrating the normal increase to be expected in the absence of a water rinse.

An analysis of the data presented shows the percentage diminution is dependent upon the number of bacteria present. Within wide limits, the nearest approach to oral asepsis occurs in patients whose mouths carry the fewest bacteria. It would therefore seem best for the dental surgeon to take advantage of this situation using antiseptic solutions at the low point in the diurnal tide or

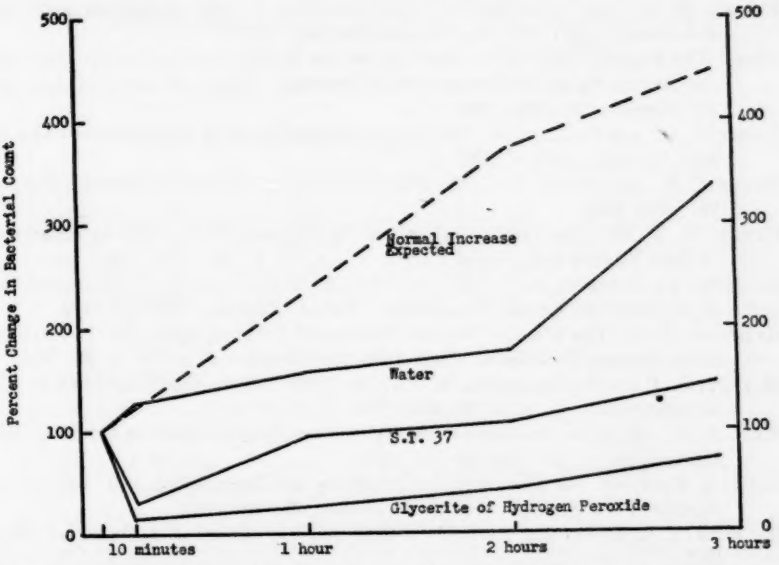


*Fig. 1.* The effect of a five minute rinse with various solutions upon the normally expected increase in the number of oral bacteria following a meal. (The Diurnal Tide)

after the number of bacteria has been reduced by antiseptic action or by additional mechanical means as by the chewing of paraffin.

## SUMMARY

The properties of glycerite of hydrogen peroxide have been listed. It has been shown to be less irritating than S.T. 37 and as causing a greater reduction in the number of oral organisms both immediately and over a longer period of time, the effects lasting up to 3 hours.

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